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A DESCRIPTION OF THE STAGES IN THE LIFE CYCLE OF THE FILARIAL WORM *LITOMOSOIDES CARINII*[1]

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The life history of the filarial worms has been known in a general way for many years, but a study of a complete life cycle in the laboratory has only been possible since *Litomosoides carinii,* a parasite of the cotton rat, became available. In connection with experiments on immunity in this species, it has been necessary to identify anatomical characters by which the degree of development of individual worms can be readily determined. These characters and certain other details of development are described in this paper. These observations in addition to those already published complete the description of all the stages and the intervening molts.

METHODS

The stages in the intermediate host were obtained from mites raised on uninfected white rats. When the mites were hungry enough to feed readily they were placed on an infected cotton rat and recovered as they dropped off 2 or more hours later. They were then kept under conditions of high humidity at temperatures controlled within a range of 5° C. The mean temperature for each series of mites was constant but varied for different series from 18° to 24° C. The infected mites were dissected in one-half strength Tyrode's solution and the larvae were measured while alive in the same solution, or in the case of the third stage larvae, after the application of sufficient heat to paralyze but not kill them.

The definitive host stages were obtained by introducing infective larvae dissected from mites into a subcutaneous pocket of an etherized cotton rat. The developing worms were recovered from saline washings of the pleural cavity of the rat. They were fixed in 70 per cent ethyl alcohol at 70° C., and after gradual changes to glycerin, were mounted in glychrogel and measured.

For brevity we shall use the term age of the worms to mean the time from entrance into the intermediate or definitive host, as the case may be.

RESULTS

Before molting to the adult stage the worms passed through the four larval stages typical of other groups of nematodes. The part of the cycle in the inter-

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mediate host conformed in general to the pattern described for several other species of filarial worms except that the variation in rate of growth of different specimens in the same host was greater than would be expected from the accounts of previous authors.

First stage larvae. Our observations lead us to the conclusion that the microfilariae have not undergone a molt and that the sheath is derived from the shell or membranes of the egg. Exactly when the term first stage larva should be applied is a matter of definition and a point we shall not discuss. The youngest larvae we studied were dissected from mites $2\frac{1}{2}$ hours after they had dropped from the rat on which they had had opportunity to feed for 2 hours. These larvae were active and similar in appearance to the larvae of *Wuchereria malayi* described by Feng (1936) after 8 hours in the mosquito, except that the posterior end of our specimens showed a longer, slenderer, and more evenly tapered portion. The length of 15 of these larvae ranged from 53 to 92 microns with a mean and standard error of 73 ± 4 microns. Their maximum diameter varied from about 5 to 6 microns. These larvae were all covered with a loosely fitting sheath.

At periods varying from 20 to 30 hours, 10 larvae averaged 94 ± 4 microns in length, ranging from 71 to 118 microns with an average maximum diameter of about 5 microns. A few were still enclosed in the sheath, a few showed no sheath, and the majority were about half way out of the sheath, the anterior end being freed first in all cases. The shape of a typical specimen of this age is shown in figure 1.

It is interesting to note that a few larvae, in no way distinguishable from these earliest forms, were frequently found in mites containing larvae in more advanced stages of development, including fully-developed infective forms at 17 days. The majority of these larvae whose development had been delayed showed no signs of a sheath, but some were entirely enclosed and others were only partially out of the sheath. Feng (1936) reported young larvae of *W. malayi* partially enclosed in the sheath at such late periods, but stated that they always showed abnormal development. In our specimens there is no evidence that delayed escape from the sheath interferes with normal development.

During the first week the larvae changed shape principally by thickening. At first this process involved only the mid-region of the worm, giving the appearance of a more sharply tapered posterior portion as seen in figure 2. By the fourth or fifh day, as in figures 3 and 4, it became evident that as the thickening process extended toward the ends, the extreme posterior portion did not change in shape. The length of 22 larvae 4 to 5 days of age ranged from 92 to 137 microns, averaging 117 ± 2 microns while the maximum diameter ranged from 6 to 15 microns, averaging 10 ± 1 micron. Usually about the sixth day, but in one case as early as the fifth, the posterior portion which does not thicken began to form a sickle shaped "tail," characteristic of the so-called sausage stage as shown in figure 5. By the seventh day about half of the larvae had the typical sausage shape as shown in figure 6. At this time the larvae were no longer actively motile, but made only sluggish movements.

The first molt. Although there were earlier indefinite signs of the development of a second cuticula, the first appearance of definite loosening of a major portion of the old cuticula appeared on the 8th day, while other larvae of the same series did not reach this stage until the 13th day and in other series none began the molt

before the 15th day. Figure 7 shows a larva in which the first molt was nearly completed. Such larvae measured from 250 to 350 by 20 to 25 microns and conformed in appearance to the larvae of *W. malayi* at the time of the first molt as shown by Feng (1936). The molt was completed in some larvae as early as the 9th day, at which time they measured on the average about 450 × 25 microns, while the majority completed this molt at various times from the 10th to the 13th day. After this molt the larvae developed rapidly as described for other species by elongating, becoming more evenly rounded at the posterior end, and again becoming more active.

The second molt. As early as the 9th day some larvae measuring from 0.350 to 0.450 mm. in length showed the cuticula of the 3rd stage well formed and that of the 2nd stage loosened at one end or the other. Figure 8 shows a larva during the process of the 2nd molt. On the 10th day some larvae had completed this molt and measured from 0.450 to 0.700 mm. in length. As in the case of earlier changes, the time of appearance of this molt was highly variable, some larvae being in the process of molting as late as the 25th day. The appearance of the latter specimens does not indicate that this delay is merely a matter of not escaping from the 2nd stage cuticula, an observation confirmed by the delayed development of other stages already mentioned. By the 13th day the majority had completed the 2nd molt and all but a few had completed it by the 15th day.

The third stage larva. After this molt the larvae underwent still further development and increased slightly in length. Because of the variability in the rate of development, it is not possible to determine the time required for this further development after the 2nd molt, but such evidence as is available indicates that it is a matter of about 2 days.

The length of 41 larvae which morphologically appeared to have reached the fully infective stage averaged 853 ± 11 microns and ranged from 509 to 965 microns. Williams and Brown (1946) and Williams (1948) stated that their larvae appeared to fall into two groups, 0.80 and 1.00 mm. in length, respectively, which they interpreted as possibly representing the two sexes. The frequency distribution of our sample does not have any distinct tendency toward a bimodal pattern, but the number examined is not sufficient to definitely rule out such a possibility.

The third stage larvae, when introduced into previously uninfected cotton rats, continued their development for about one week before they showed signs of molting. At the end of this period 21 larvae averaged 0.94 ± 0.023 mm. in length, representing a small but statistically significant increase over the length of the infective larvae. In most specimens the reproductive system showed distinct signs of development at this time. The vulva was usually well rounded and had a lumen, but there was no opening through the cuticula. The connecting oviduct was generally clearly visible. The cloaca was usually distinct and an indication of early development of the spicules was often present, but the latter showed no signs of sclerotization.

The sclerotized stoma is the most characteristic feature by which the third and fourth larval stages and the adult stage may be distiguished. That of the third stage appears in figure 9 where it is shown attached to the molting cuticula. It does not change in size or appearance from the time it forms during the second molt in the mite until it is cast off at the third molt. It is readily recognized by its slender

tapering shape and especially by the two highly refractile dots representing optical sections of the anterior ring.

The third molt. The first sign that the larva is preparing to molt is the appearance of the fourth stage stoma within the cuticular wall, but around the invaginated third stage stoma. Soon thereafter the new cuticula can be seen within the old one and separation of the two may begin at either end. The old cuticula is usually completely loosened before the old stoma pulls out of the new one. The anterior end of a larva in the process of molting is shown in figure 9. The break in the old cuticula through which the larva escapes may occur at any place. At 8 days after infection, 11 per cent of 63 worms from 4 rats still showed no signs of molting and 16 per cent had completed the molt and left the old cuticula. At 9 days after infection 56 per cent of 34 worms from 2 rats had completed the molt and all at least showed signs of molting. At 10 to 15 days after infection, 88 per cent of 52 worms from 4 rats had completed the molt. In the 29 worms which had completed the molt on the 8th and 9th day the mean length was 1.20 ± .03 mm. In other words, approximately a 25 per cent increase in length occurred during the molting process.

The fourth stage larvae. Considerable growth occurred during the fourth stage which lasted on the average until about 24 days after infection. The mean length of 17 male worms, which showed no signs of molting when recovered from 5 rats killed 23 or 24 days after infection, was 6.42 ± .52 mm., while the mean length of 43 similar female worms was 8.76 ± .24 mm. The internal development during this stage has been described by Cross and Scott (1947). A study of these additional specimens shows that the vulva is apparently patent at the end of this stage, judging from the appearance of the sclerotized portion of its lining which is freed with the cuticula at the molt. The characteristic sclerotized stoma of this stage is shown in figure 9. As in the third and adult stages this structure does not change in appearance or size during the entire stage. It is easily seen in most specimens and readily differentiated from the third stage stoma by its parallel sides and from the adult stoma by its relatively long, slender form. In 25 worms immediately before the final molt the stoma averaged 91 ± 1.9 microns in length as compared with 63 ± 1.6 microns, the average length of the adult stoma of 18 worms immediately after the final molt.

The final molt. As in the previous molt the first sign of molting is the appearance of the new, sclerotized stoma surrounding the old one. The molt is again completed by the loosening of the old stoma after the rest of the cuticula has separated. The other phases of this molt have already been described by Cross and Scott (1947). The male worms, although smaller than the females, molt at a slightly earlier date. Of 82 males recovered from 5 rats 23 or 24 days after infection, 21 per cent had not started to molt, while 41 per cent had completed the molt. In comparison, 52 per cent of 83 females from the same rats had not started to molt and only 4 per cent had completed the molt. An increase in length of 1 or 2 mm. occurs during the molt and rapid growth in length of the adult stage begins immediately thereafter.

DISCUSSION

It is believed that the observations reported here complete for the first time a description of all the developmental stages and the intervening molts of a member of the FILARIOIDEA. The microfilariae and the adults have been described in detail

for many species. The larval stages in the intermediate host have been described for several species including the relatively detailed descriptions of *Wuchereria malayi* by Feng (1936), *W. bancrofti* by Abe (1937) and of several filariae of frogs by Kotcher (1941). The only species other than *Litomosoides carinii* for which early definitive host stages have been described is *Conispiculum flavescens,* a filaria of the lizard. Pandit, Pandit and Iyer (1929) described a series of larval forms of this species developing in *Culex fatigans.* Menon, Ramamurti and Rao (1944) were able to produce infections in the lizard and described the developing worms at various ages. Neither of these two groups of authors, however, mention seeing the molts, nor do their figures or descriptions make possible the identification of the successive typical larval stages.

The microfilariae of *L. carinii* were described by Bell and Brown (1945) who also give references to the less complete descriptions of the earlier authors. Kershaw (1949) described changes in the microfilariae which occur as they pass into the peripheral circulation of the cotton rat. Williams and Brown (1945) and Williams (1948) briefly described certain typical stages developing in the mite. Bertram (1947) also briefly described certain stages in the mite, giving their size and the relation to age and temperature. It appears that the observations reported here represent the first attempt to trace the development of this species in the mite at sufficiently frequent intervals to follow the complete sequence of development. Our description of the third molt, added to the information already provided by Cross and Scott (1947), completes the basic knowledge of the life cycle within the definitive host. Thus all of the developmental stages in the life cycle of this species have been described, although many anatomical details are still in need of further study.

On the basis of the evidence available that the microfilaria develops directly from the embryo to the first stage larva without a molt, this species follows the pattern of other nematodes, i.e., three larval stages prior to entrance into the definitive host, the last of which is transmitted to that host; a third molt within the definitive host results in a fourth stage larva which later molts into the adult stage. The only evidence opposed to the acceptance of this similarity to other nematodes is that offered by Augustine (1937 a and b) and earlier authors who contended that the sheath on the microfilaria represents the cuticula of a first stage larva and that the microfilaria should, therefore, be considered a second stage form. Kotcher (1941) reviewed the evidence and concluded that this interpretation cannot be supported. Evidence from the study of *L. carinii* leads us to the same conclusion. Moreover, it would seem logical to expect this group to conform to the pattern of other nematodes.

Experimental studies of Bertram (1947) showed that larvae developing at 23–25° C., become infective at about the 15th day. The present studies at the same or slightly lower temperatures show that the majority reach the full development of the infective form at about 15 days and nearly all by the 17th day. After development at these same temperatures, our colonies routinely dissected for experimental purposes showed a majority of the larvae to be infective on the 15th day and nearly all had attained the maximum morphological development by the 20th day. We have some evidence to indicate that larvae which have completed the second

molt but have not completed the morphological development usually occurring in the mite, may already be infective if they are artificially transferred to the rat.

In general the measurements of the intermediate host stages given here agree with those given by previous authors, although it is difficult to compare them in detail. Measurements vary considerably with the conditions under which they are made and some authors have not given very precise information regarding the important relation of age and temperature. Our measurements cannot be considered as definitive because of the variability of growth rate but give a good indication of the status at typical stages in the growth.

The variability in the rate of development in the intermediae host cannot be satisfactorily interpreted at the present time. Larvae developing at a delayed rate give every appearance of normal development. Dead larvae which are occasionally found are usually at a stage of development corresponding to that of the majority of live larvae in the same mites. On the other hand, as shown by Bertram (1950) the number of larvae developing in different mites fed simultaneously on the same rat is highly variable and not entirely correlated with the size of the blood meal, many mites having no larvae after feeding on rats with a high microfilarial count.

SUMMARY

A description of the stages of *Litomosoides carinii,* filarial parasite of the cotton rat, occurring in the tropical rat mite, *Bdellonyssus bacoti,* shows that they are essentially similar to those previously described for related species. The first molt occurs at about 9 days and the second at about 13 days at temperatures averaging 18 to 24° C. The third stage larva usually attains its maximum development in the mite in another 2 days and when transferred to the cotton rat continues to develop for about a week with slight increase in size. The molt from the third to the fourth stage is described for the first time. Considerable growth occurs during the fourth stage and the fourth molt is reached in about 24 days. The sclerotized stoma is a characteristic feature by which the third, fourth and adult stages can be distinguished. Since this is the first species among the filarial worms for which all stages and molts are described, it is of interest that the typical stages of the life cycle conform to those of other nematodes.

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PLATE I

FIG. 1. Larva 0.104 mm. long in embryonic sheath, from mite dissected 18 hours after removal from an infected cotton rat on which it had opportunity to feed for 4 hours.

FIG. 2. Larva 0.107 mm. long in embryonic sheath, 4 days after feeding period.

FIG. 3. Larva 0.110 mm. long without sheath, 5 days after feeding period.

FIG. 4. Larva 0.145 mm. long in embryonic sheath, 7 days after feeding period .

FIG. 5. Larva 0.163 mm. long without sheath, 8 days after feeding period; drawn to smaller scale.

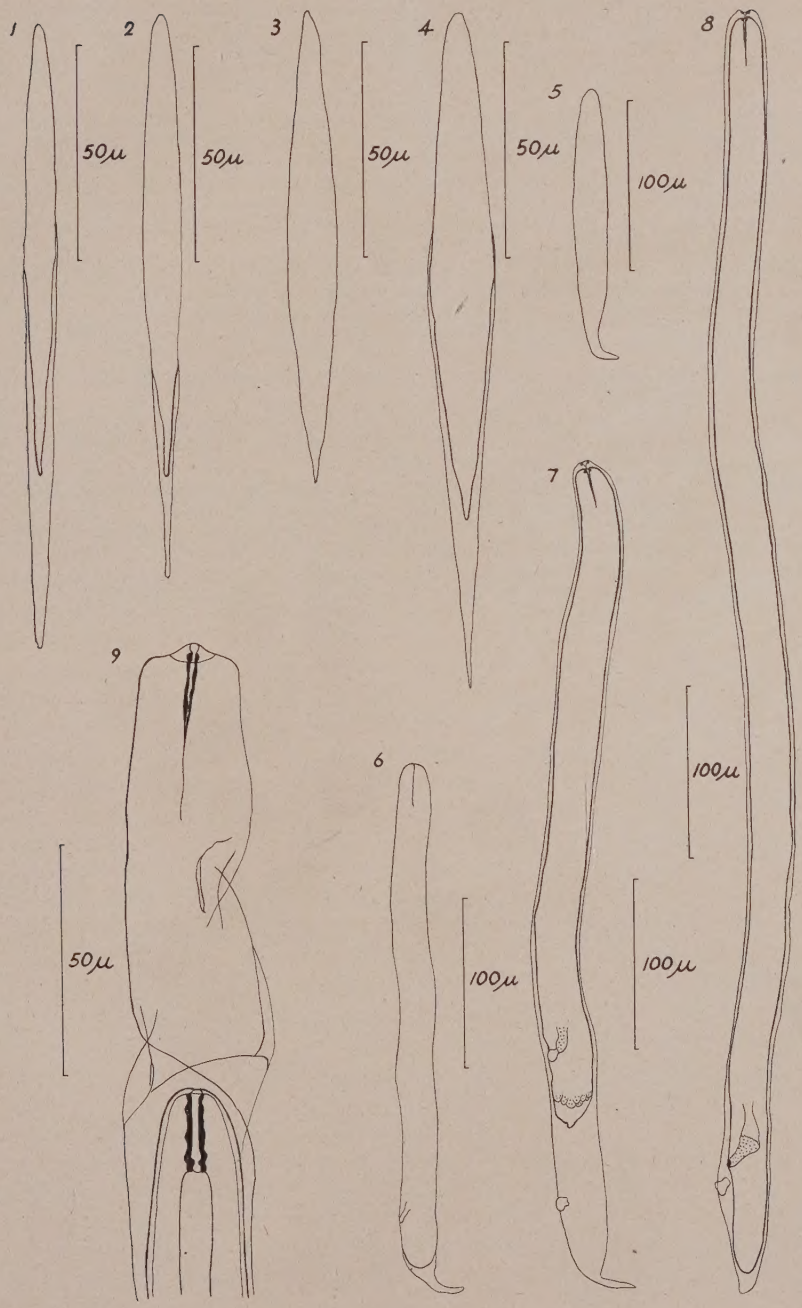
FIG. 6. Larva 0.310 mm. long, 9 days after feeding period.

FIG. 7. Larva 0.390 mm. long, during first molt, 10 days after feeding period.

FIG. 8. Larva 0.740 mm. long, during second molt, 11 days after feeding period.

FIG. 9. Anterior end of fourth stage larva shedding cuticula of the third stage.

PLATE I



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RECENT OBSERVATIONS OF CANNIBALISM IN *TRIATOMA* (HEMIPTERA: REDUVIIDAE)

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In 1914 E. Brumpt noticed in several of his colonies unfed triatomid nymphs feeding on engorged nymphs. The species reported engaging in this type of what he called "cannibalism" were *Triatoma infestans* (Klug), *T. chagasi* Brumpt & Gomez, *T. sordida* (Stål), *Mestor megista* (Burmeister) and (plus coprophagous feeding) *Rhodnius prolixus* Stål. Brumpt then pointed out that *Trypanosoma cruzi,* the etiologic organism of Chagas' disease, could be transmitted from infected nymphs to those uninfected and might be a means of greatly increasing the percentage of infection in the invertebrate host in nature. Since Brumpt's report in 1914, little mention has been made of this phenomenon.

On April 24, May 5, 12 and 14, 1950, and on all feeding periods subsequent to this date *Triatoma phyllosoma pallidipennis* (Stål) has actively engaged in cannibalism in the colony under observation. *T. guasayana* Wygodzinsky and Abalos, *T. longipes* Barber and *R. prolixus* have also been observed feeding in this manner, but not nearly as actively as *T. phyllosoma pallidipennis.* In all species mentioned above cannibalistic feeding was limited to the nymphs. On several occasions unfed nymphs attempted to pierce the adult integument but without success.

The following notes were recorded on May 14, 1950 and are typical of all sub sequent observations. Usually young unfed nymphs are observed feeding on the older well-engorged nymphs; however, this is not always the situation. Cannibalism does not occur until newly engorged nymphs move down from the mammalian host and pass near the unfed members of the colony. Older instar nymphs of *T. phyllosoma pallidipennis* are able to ingest considerable warm blood from the host and sufficient heat is retained by them to stimulate a probable thermotropic response from unengorged nymphs. Herein then would seem to lie the reason for this generalized probing of newly engorged nymphs by those that have not yet fed. The older nymphs of the colony crowd up to the host to the exclusion of the younger members of the colony; this would seem to explain the fact that younger nymphs feed on fourth and fifth instars to a greater extent than older nymphs feed on the second and third instars. As many as five first instar nymphs have frequently been observed crowding about a fourth or fifth instar nymph and from one to three ultimately insert their beaks into the victim's abdomen. In a few minutes these nymphs have been seen to partially engorge themselves with blood drawn from the blood-filled intestine of their freshly fed, fellow colony mate.

Victims of this type of feeding do not seem to suffer any ill effects, since droplets of blood do not appear externally from the punctures and no nymphs have been found dead as a result of this type of cannibalism. Those individuals being fed upon show some annoyance but remain quiet for long enough periods to allow feeding by cannibalistic members. Fourth instar nymphs have been observed pulling

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small nymphs about, attached only by the latter's probocides inserted into the abdomen of the former. The feeding nymphs remain attached until a substantial blood meal has been acquired at which time they have little difficulty in releasing themselves. Separation seems easily effected when the feeding nymphs retract the barbed mandibles into the labium.

Cannibalism is not an accurate, descriptive term for the type of feeding practiced by *Triatoma* bugs under certain conditions; however, it has been used since 1914 for the lack of a better term. A new term, *"kleptohemodeipnonism,"* is proposed to describe this unique feeding habit which is frequently practiced by members of the Triatominae. Literal translation of *klepto-hemo-deipnon-ism* is the theft of a blood meal.

The above observations were made while the author was a student at the University of California in Berkeley.

I am indebted to Dr. R. L. Usinger and Dr. B. W. Halstead for many helpful suggestions in composing this paper.

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ECHINOCEPHALUS PSEUDOUNCINATUS N. SP., A NEMATODE PARASITE OF THE ABALONE*

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Although the shells of abalones are frequently damaged by boring organisms, there is only one report in the literature of the occurrence of endoparasites in *Haliotis*. That case was reported by D. R. Crofts (1929) in the species *H. tuberculata,* an inhabitant of the English Channel. She found one adult female in a shrunken condition parasitized by an adult trematode; sporocysts, rediae, and cercariae were also present. One male abalone also contained a number of haplosporidian cysts located between the digestive gland and the testis.

The writer has examined thirty specimens of the pink abalone (*Haliotis corrugata*) of Southern California and found them to be heavily infected with a larval nematode parasite belonging to the superfamily SPIRUROIDEA, the family GNATHOSTOMIDAE, and the genus *Echinocephalus*. Only old abalones were found to harbor the worms; these encyst in the ventral portion of the foot producing a blister-like effect on the outside of this structure. This vesicatory effect and the burrowing of the larvae through the foot prior to encystment, apparently weakens the muscle and decreases the efficacy of this structure as a hold-fast organ. These two salient characteristics of an infected abalone, the appearance of the foot and the ease with which the animal may be removed from the rocks, enable the commercial divers to distinguish between the "healthy" and the infected animals. Aside from the above mentioned effects the host seems to suffer no other ill effects from the parasite.

A few specimens of the southern green abalone (*H. fulgens*) were also examined, but were found to be free from this parasite. However, reliable reports from the divers and the processing plants indicate that the green abalone is as susceptible to this parasite as is the pink abalone, an indication that the parasite is not host specific.

Echinocephalus pseudouncinatus n. sp.

(Fig. 1 & 2)

Description (Based on fifty-five specimens). Length 18.64 mm.; thickness 0.6388 mm.; length of head 0.3488 mm.; width of head 0.4455 mm.; length of hooks 0.0273 mm.; distance between rows of hooks 0.0289 mm.; distance between striations of the body 0.0135 mm.; distance from head end to end of cervical sacs 1.95 mm.; distance from head end to end of esophagus 3.08 mm.; distance from tip of tail to anus 0.17 mm.; width of lips 0.1194 mm.

The head-bulb has 6–6½ rows of hooks with 40–50 hooks per row. The hooks increase in size from anterior to posterior, and the dorsal and ventral hooks in each row are smaller than the lateral ones in the same row (Fig. 2). There are two very incomplete rows of rudimentary hooks surrounding the lips (Fig. 2). Each

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circular row of hooks is interrupted laterally by a hookless area as shown in Fig. 2. Two lips are present, each lip bearing two central papillae. The lips have no teeth or cutting ridges. The striations of the cuticle are distinct and fine. A single minute postanal papilla was present in the majority of the worms, but no cervical papillae or alae were detected. Phasmids, nerve ring, and excretory system were not observed. Since these are immature worms, bursae, spicules and reproductive

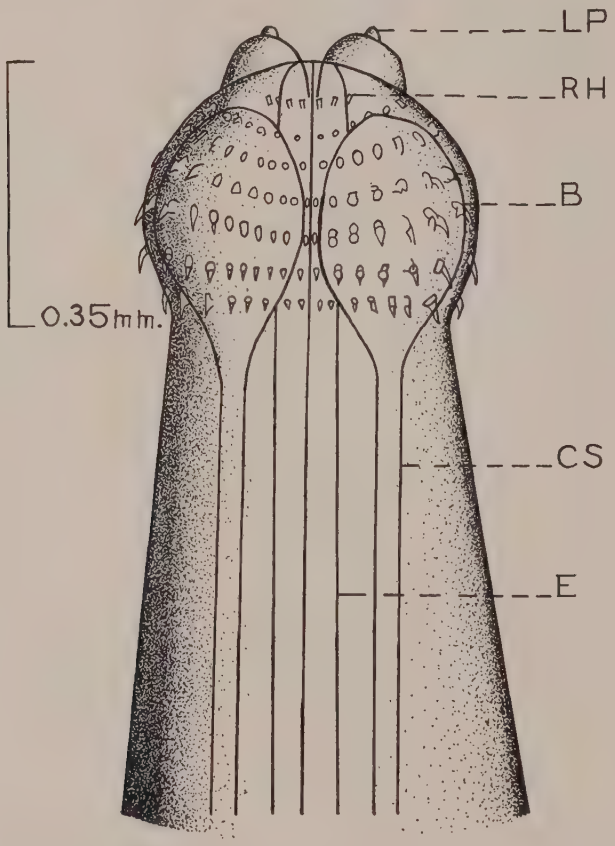

FIG. 1. Free-hand drawing. ×128. Dorsal view of *E. pseudouncinatus* showing the pair of lips, six rows of hooks, and an incomplete row of rudimentary hooks around the lips.

Abbreviations

B	ballonet
CS	cervical sac
E	esophagus
LP	lip papilla
RH	rudimentary hooks

organs were not observed. The esophagus and the intestine are well developed, and occupy almost the entirety of the pseudocoelom. Five-sixths of the digestive system consists of intestine. The intestine conforms to the typical nematode pattern, being made up of simple columnar epithelium bearing on its internal surface a "ciliated" bacillary layer, followed by a distinct subbacillary layer adjacent to the

cytoplasm. The external surface of the cells is covered by a discrete basal lamella. The esophagus is 1/6 of the body length, is triradiate, one ray of the lumen is directed ventrally, and each ray is connected to the margin by fibers or radii. Between these radii are the radial muscles, and the esophagus is covered externally by a semicuticular membrane. The anterior portion of the esophagus is short and muscular, while the posterior part is longer, wider, and more glandular. The anus is ventral and subterminal. Four ballonets are located in the head-bulb and divide the latter into quadrants (Fig. 1). Each ballonet at the junction of the head-bulb and the body decreases in diameter to form a cervical sac (Fig. 1). The latter continue as ducts approximately 2/3 the distance of the esophagus where each one terminates in a button-like appendage.

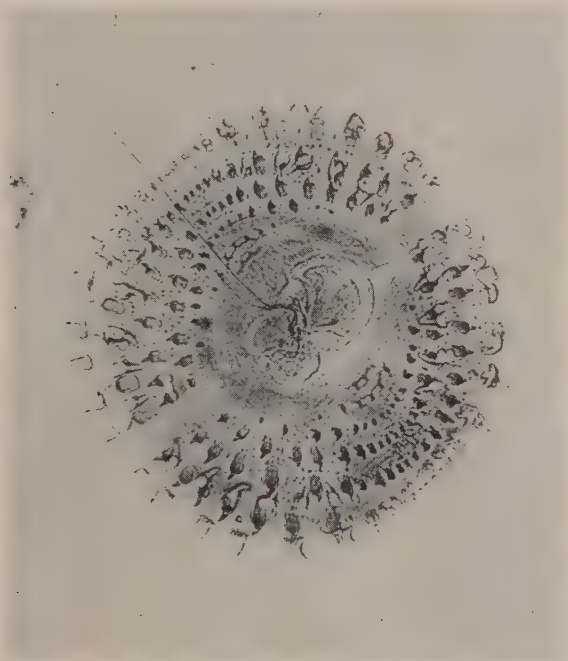

FIG. 2. Photomicrograph. ×128. En face view of *E. pseudouncinatus* showing lips, rudimentary hooks, decrease in size of hooks dorso-ventrally, and the lateral separation.

Host: Haliotis corrugata, pink abalone

Location: encysted in the foot

Locality: Pyramid Cove, San Clemente Island, Los Angeles County

Discussion: As presently constituted, the genus *Echinocephalus* includes four definite species: *E. uncinatus* Molin (1858), *E. southwelli* Baylis and Lane (1920), *E. multidentatus* Baylis and Lane (1920), and *E. spinosissimus* von Linstow (in Shipley and Hornell, 1905). Monticelli (1889) gave the name *E. striatus* to some specimens he found in the stomach of *Scyllium* sp. from Payta, Peru. There is no means of identifying his species, and the name is considered by Baylis and Lane (1920) to be a *nomen nudum*. The size of the hooks on the head-bulb, the number

of rows of hooks, and the number of hooks in each row are good specific characters. The presence or absence of teeth on the lips and their number are also reliable diagnostic characteristics.

E. southwelli, E. multidentatus and *E. spinosissimus* have more than 7 rows of hooks on the head-bulb and more than 100 hooks per row, whereas *E. uncinatus* has six rows of hooks and 40–50 hooks per row. In this respect the parasite of the abalone most nearly resembles *E. uncinatus.*

However, in comparing the worms from the abalone with the description of *E. uncinatus* found by Baylis and Lane (1920) in *Pinna* sp. and in the sting-ray (*Myliobatis nieuhofi*) from Ceylon, significant differences become apparent. The worms from the abalone are more than four millimeters longer, and are thicker by 0.2 mm., but the hooks are 0.02–0.03 mm. shorter, and the distance between the rows of hooks is smaller by 0.03–0.04 mm. In the specimens of Baylis and Lane the hooks in each row are the same size, and in each row the hooks form a complete circle, whereas, in the worms from the abalone the dorsal and ventral hooks in each row are smaller than the lateral ones, and each circular row of hooks is interrupted laterally by a hookless area.

The lips were not developed in any of the specimens of Baylis and Lane, but von Linstow (in Shipley and Hornell, 1904) stated that the specimens of *E. uncinatus* found by him in the pearl oyster (*Margaritifera vulgaris*) of Ceylon, had six lips on the head, while those from the abalone have two undivided lips.

For these reasons, it is proposed that the worms from the abalone be referred to a new species. *E. pseudouncinatus* is suggested as the name for this new species because it implies that the worms are similar to those of the species *uncinatus* and yet are different enough to be considered as a distinct species.

Prior to its discovery in the abalone, the only specimen of *Echinocephalus* found in North America was the one discovered by Van Cleave in the gonad of the sea urchin, *Arbacia punctulata* taken at Woods Hole in May 1917. It was described by Hopkins (1935) and tentatively assigned to the species *E. uncinatus.* This specimen was obtained on loan from the U. S. National Museum and its measurements were recorded. The worm was originally fixed in formaldehyde, which from the writer's experience does cause shrinkage of the more labile parts, especially the head. However, the more fixed structures such as the hooks will not be subject to such shrinkage. As an added complication, the specimen was spirally coiled, making examination and measurements more difficult.

The worm was externally morphologically identical with the ones from the abalone. There were six rows of hooks and approximately forty hooks per row. Of more significance was the decrease in the size of the hooks dorso-ventrally as compared with the lateral ones in the same row, a situation identical with that found in the abalone parasites. No teeth were present on the pair of undivided lips.

The head is smaller, both in length and width, than the specimens found in the abalone, or in the specimens of Baylis and Lane. The lips are also smaller than the lips of the worms from the abalone. These differences may be due to the shrinkage caused by the formaldehyde. The worm is intermediate in thickness between the other two worms, but if allowance is made for shrinkage then Van Cleave's specimen approximates the worms from the abalone.

The length of the hooks 0.0236 mm. and the distance between the rows of hooks

0.0307 mm. agree more closely with the measurements of the worms from the abalone (0.0273 mm. and 0.0289 mm. respectively) than they do with the specimens of Baylis and Lane (0.0475–0.055 mm. and 0.045–0.06 mm. respectively).

It is, therefore, suggested that Van Cleave's specimen be reassigned to the new species, *E. pseudouncinatus.*

Not too much is known about the life cycle of the species of *Echinocephalus.* Adults of the three species *E. spinosissimus, E. southwelli,* and *E. multidentatus* have been found in the spiral valve region of the intestine of sting-rays of Ceylon and the Adriatic. Molin (1858, 1861) found adult *E. uncinatus* in the large intestine of the sting-ray, *Trygon brucco,* in the Adriatic. von Linstow (1904) also found the adult in the sting-rays, *T. brucco, T. pastinaca.* Immature forms of *E. uncinatus* have been found by Baylis and Lane in the sting-ray, *Myliobatis nieuhofi* of Ceylon, and by Shipley and Hornell (1904) in the trigger fishes, *Balistes mitis, B. stellatus* of Ceylon. Larvae of *E. uncinatus* have been described from the mollusc, *Pinna* sp. by Baylis and Lane, and from the pearl oyster, *Margaritifera vulgaris* of Ceylon by von Linstow.

The evidence indicates, therefore, that this parasite requires a mollusc as an intermediate host upon which the final hosts feed. It is said that the species of *Trygon* feed upon both the pearl oyster and the trigger fishes, however, it is not clear whether the trigger fish serves as a second intermediate host or whether the presence of the worms in this fish was abnormal. Its occurrence in the sea urchin as reported by Hopkins, may be purely accidental.

The California sheepshead, *Pimelometopon pulchrum* a teleost fish, is the chief enemy of the abalone. The author so far, has examined two specimens and although other nematodes were present no adult *Echinocephalus* could be found.

The only other possible enemies of the abalone are the elasmobranch fishes, the rays. Specimens of the round sting-ray, *Urobatis halleri,* and the shovelnose guitarfish, *Rhinobatos productus* were examined and found to be free of any parasites.

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HOST-TISSUE REACTIONS TO INITIAL AND SUPERIMPOSED INFECTIONS WITH *HYMENOLEPIS NANA* VAR. *FRATERNA*[1]

W. S. Bailey[2]

The marked protection induced in the rodent host by infections with *Hymenolepis nana* was noted by Grassi (1887) and later by Joyeux (1920) who concluded that one infection in rats made a second one impossible. Studies by Shorb (1933), Hunninen (1935), and Hearin (1941) have clearly demonstrated the effectiveness of this immunity in preventing subsequent infections. Although Hearin (1941) and Larsh (1943) demonstrated the antibody basis of the acquired immunity, no studies have been reported on the specific mechanism of this immune response. In this study, mice from the laboratory colony were given one or two egg infections of *H. nana* var. *fraterna,* and tissue sections of the small intestine were studied in an effort to obtain information on the tissue reactions and on the fate of the oncospheres in second infections.

MATERIALS AND METHODS

All tissues were fixed in 5 per cent formalin in physiological saline and embedded in paraffin by coiling the piece of intestine to be sectioned so that a longitudinal section of the full length would be obtained. Serial sections 6 microns in thickness were cut, and routine haematoxylin and eosin staining was employed. The entire section on the slide was examined in searching for larvae in the villi. In some of the mice it was important that the total number of cysticercoids present in a given number of serial sections be determined. This number was arrived at by plotting on an outline drawing of the specimen being examined the location of each cysticercoid found in each section.

TISSUE REACTIONS IN INITIAL INFECTIONS

Mice approximately 2 to 2½ months of age were given an initial egg infection and killed at approximately 24-hour intervals. The small intestine was removed immediately, and that portion where the cysticercoids develop in largest numbers (the 10th to 20th cm.) was removed and sectioned.

In tissue sections from mice killed 24 hours after infection, larvae had reached the tunica propria of the villi. At 48 hours they had increased somewhat in size; no cellular infiltration was recognizable in the sections at this time. The steady growth of the larvae caused a corresponding displacement of the host tissue cells, and a cellular differentiation in the developing larvae was seen in some sections at the end of 72 hours. By this time a slight cellular infiltration was seen (Fig. 1). Growth and differentiation proceeded at a rapid rate during the next 24 hours, and

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at 96 hours the cysticercoids were completely developed (Fig. 2). At this time cellular infiltration had become rather marked. Most of the infiltration was confined to the area around the base of the villus extending into the tunica propria; only a relatively few of the cells had migrated around the parasite toward the tip of the villus. The predominant type of cell was the polymorphonuclear leucocyte, but some lymphocytes were also present. Many of the polymorphonuclear cells contained heavy granular acidophilic cytoplasm characteristic of eosinophiles. There was no noticeable increase in fibrocytes and no evidence that a tissue wall was being formed around the parasite.

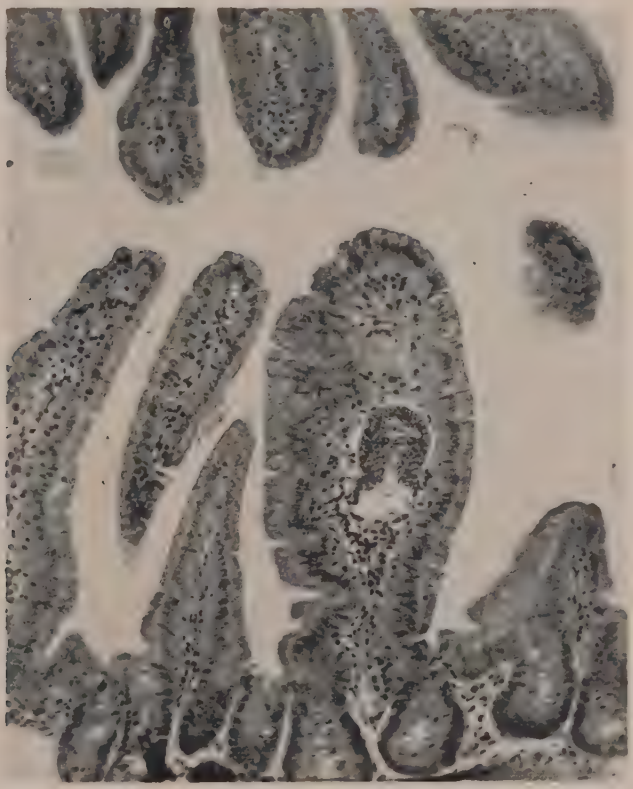

FIG. 1. Section of small intestine of mouse showing cysticercoid of *H. nana* var. *fraterna* 72 hours after infection (145×). (Negative No. 287221-2, Armed Forces Institute of Pathology.)

FATE OF ONCOSPHERES OF A SECOND INFECTION

An effort was made to determine the fate of the oncospheres of a second infection by examining sections of the intestines of mice that had been given 2 infections. In the first experiment 3 mice were each given 8,850 eggs 5 days after an initial infection with 21,300 eggs. They were killed 24, 48, and 72 hours after the second infection, and serial sections made from the second 10 cm. of the small intestine were examined for the presence of developing larvae. As shown in Table 1, none were found to have penetrated in any of the serial sections examined.

Since in this experiment there were no controls receiving only infection II, a second experiment was conducted in which 3 mice received an initial infection of 18,500 eggs each and a second infection of 21,300 eggs each on the 10th day. At the same time a previously uninfected mouse received infection II. The test animals were killed 18, 48, and 72 hours after infection II and 32, 13, and 14 serial sections from the second 10 cm. of their intestines examined for larvae. As shown in Table 1, no larvae were found in any of these sections. The control mouse (T-21) was killed 96 hours after infection, and in 14 serial sections of the same portion of its intestine a total of 51 larvae was found within the mucosa.

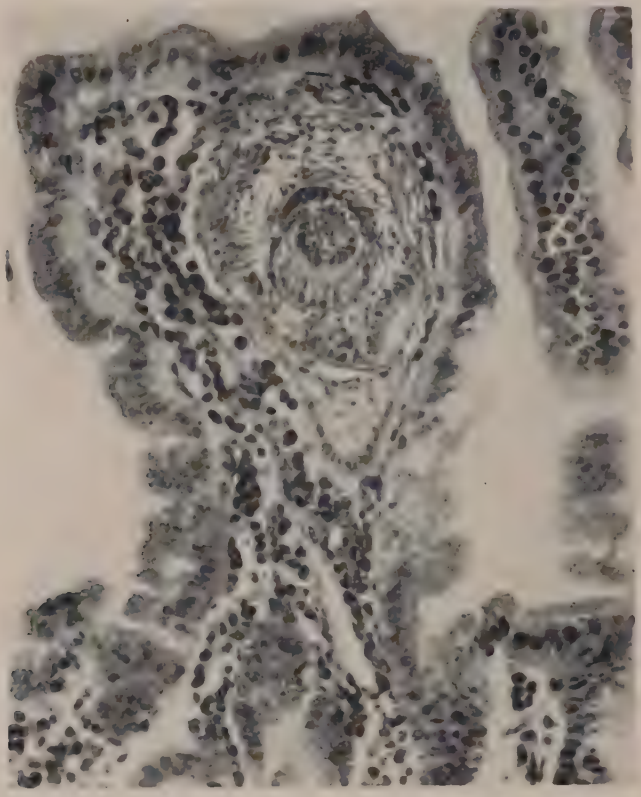

FIG. 2. Section through a fully developed cysticercoid of *H. nana* var. *fraterna* 96 hours after infection (395×). (Negative No. 287230-1, Armed Forces Institute of Pathology.)

In a third experiment performed to check these results 2 mice each received a second egg infection of 31,500 eggs 5 days after an initial infection with 1,430 eggs. Two control mice received only infection II. One control and one test animal were killed 28 hours after infection II and the other pair at 50 hours. The results are shown in Table 1. In 10 serial sections from the control mouse (T-25) killed 28 hours after infection 24 larvae were found. In a like number of sections from the corresponding test animal (T-23), 8 larvae were found. The 10 serial sections from control mouse (T-26) contained 30 larvae, while the corresponding test animal (T-24) showed no larvae in 10 sections.

Polymorphonuclear leucocytes, some of which were eosinophiles, were found in the connective tissue surrounding the larvae which had penetrated the mucosa of the previously infected mouse (T-23). This cellular infiltration occurred earlier than in mice with an initial infection.

DISCUSSION AND CONCLUSIONS

The results of these experiments, although the number of animals used was small, strongly suggest that most of the oncospheres of a second egg infection with *H. nana* var. *fraterna* are unable to penetrate the mucosa of the small intestine. Of 8 mice given a second egg infection 5 or 10 days after the initial one and killed after 18 to 72 hours, larvae from the second infection were found to have penetrated the mucosa of the intestine in only one mouse. The number present in this mouse was smaller than that found in the control mouse. It is probably significant that this mouse was one of the two with the smallest initial infection and the largest second infection. It may also be significant that a more rapid cellular reaction had been

TABLE 1.—*Number of larvae found in resistant and susceptible mice after two infections with eggs of* H. nana *var.* fraterna

Test animals (Infections I and II)				Control animals (Infection II only)			
Mouse no.	Hours after infection	Number of sections examined	Total number larvae	Mouse no.	Hours after infection	Number of sections examined	Total number larvae
Experiment I: Initial infection 21,300 eggs; infection II 8,850 eggs on 5th day.							
T-4	24	10	0				
T-5	48	10	0				
T-6	72	10	0				
Experiment II: Initial infection 18,500 eggs; infection II 21,300 eggs on 10th day.							
T-15	18	32	0	T-21	96	14	51
T-16	48	13	0				
T-17	72	14	0				
Experiment III: Initial infection 1,430 eggs; infection II 31,500 eggs on 5th day.							
T-23	28	10	8	T-25	28	10	24
T-24	50	10	0	T-26	50	10	30

elicited around the few larvae that had penetrated the intestine of this mouse from a second infection than was found in mice following initial infection.

Leonard (1940) advanced the hypothesis that the intestine or intestinal wall becomes a serious barrier to oncospheres of *Taenia pisiformis* in superimposed infections in rabbits. Leonard and Leonard (1941) presented conclusive evidence in support of this hypothesis and concluded that the resistance against the larvae in immune rabbits exists in two distinct phases, intestinal and parenteral. Since they found that penetration of the oncospheres was very rapid, they concluded that their destruction in the lumen was unlikely and that it probably occurred after penetration of the mucosa as the result of a tissue reaction.

The conclusion that most of the oncospheres of second infections of *H. nana* var. *fraterna* are unable to penetrate the mucosa is given strong support in the demonstration by Hearin (1941) that the protective resistance against this parasite develops within 12 hours. It is interesting that an immune response could prevent invasion of the mucosa of new larvae while at the same time not interfere with the development in situ of others just 12 hours older. Another point with reference to

the time relationships seems significant. The available information on the immune response against other helminth parasites suggests that several days are required for the development of a reaction capable of completely inhibiting, walling off, or killing the parasite. For example, Leonard (1940) found the first evidence of arrested development in the larvae of *T. pisiformis* in passively immunized rabbits on the 4th day after infection, and dead larvae were first found on the 6th day. Since only 93 hours are required for the complete development of the cysticercoids of *H. nana* var. *fraterna,* it would appear that, if the principal factor in the immune response to superimposed infection were the usual antibody-antigen and host-tissue reactions, the larvae would not be overcome before most of this growth had occurred. Thus, many dead or encapsulated larvae would be noticeable on the examination of the small intestine for cysticercoids.

On the basis of the results of these experiments it is suggested that the immunity against *H. nana* var. *fraterna* like that to the larvae of *T. pisiformis* exists in more than one phase. The great majority of the oncospheres apparently are unable to penetrate the mucosa of the small intestine of previously infected animals. It seems probable that a second phase exists in which an accelerated host-tissue response is capable of overcoming the few larvae which are able to gain access to the tunica propria of the villi. The actual mechanism operative in preventing the penetration of the oncospheres in resistant animals infected with *H. nana* var. *fraterna* is not yet apparent. It seems likely that the initial infection produces some change in the epithelial tissue itself which renders penetration difficult or impossible, or that some action is exerted on the embryos in the lumen of the intestine which renders the majority of them incapable of penetration.

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OBSERVATIONS ON THE TRICHOMONAD FLAGELLATE OF THE REPRODUCTIVE ORGANS OF CATTLE

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INTRODUCTION

In 1945 I obtained bacteria-free cultures of the trichomonad in the reproductive organs of cattle from Dr. B. B. Morgan. They were maintained for several months, studies were made of living flagellates, and slides were prepared. Some slides were stained by the Heidenhain technique and others impregnated by the Bodian protargol procedure. Some observations on abnormal displacement of flagella and the undulating membrane have been reported (Kirby, 1947). Studies on the structure of the flagellate and the division process are dealt with in this paper. It is not intended that this should be a complete account, because an accurate and extensive description of the features revealed by the usual techniques has already been given by Wenrich and Emmerson (1933). I am able to supplement their account in some respects as a result of dark-field observations and studies of flagellates impregnated by the protein silver technique.

Acknowledgments are made to Dr. Morgan, who supplied the strain used for these studies; to Dr. Bronislaw Honigberg, who aided in maintaining the cultures and preparing material; to Dr. E. N. Kozloff, who prepared some slides used in the study; and to Mrs. Lois C. Taylor, who made the line drawings and aided in the observational work. The dark-field drawing and the photographs were made by the author.

NOMENCLATURE

The trichomonad associated with sporadic abortion in cattle was named *Trichomonas foetus* by Riedmüller (1928). It had previously been named *Trichomonas utero-vaginalis vitulae* by Mazzanti (1900). Riedmüller noted that Mazzanti's description did not make possible a comparison with the trichomonad he studied. Although there is no doubt that Mazzanti dealt with the one trichomonad that occurs in the vagina and uterus of cows, the name he proposed is not in proper nomenclatural form, and has not since been used except in synonymy.

Morisita (1939) remarked that the next name having priority is *Trichomonas fetus* as used by Hopfengärtner et Ernst, 1924. He noted that Drescher in 1925 first communicated this finding by Ernst and Hopfengärtner. So far as I have been able to find out, from Morisita's paper and otherwise, there was no publication of the name by the authors to whom it was attributed. Because Riedmüller's name has been generally used in an extensive literature for more than twenty years, and because in any case the status of the name attributed to Hopfengärtner and Ernst is uncertain, it is undesirable to try to apply equivocal priority.

As the generic name, *Trichomonas,* has been used by many authors and *Tritrichomonas* by some. Wenrich and Emmerson (1933) wrote *Tritrichomonas foetus* (Riedmüller), and were evidently responsible for the first combination of those generic and specific trivial names. But Wenrich (1935 and subsequently) used *Trichomonas* instead. On the other hand, Morgan (1944 and elsewhere) had

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used the name *Trichomonas foetus,* but Morgan and Hawkins (1948) adopted *Tritrichomonas* instead.

The type species of *Tritrichomonas* Kofoid, 1920 is *T. augusta* Alexeieff, 1911, the only species Kofoid mentioned in the genus he proposed. Except for the presence of only three flagella, the characteristics given in Kofoid's diagnosis of the genus are not significant for taxonomic differentiation within the TRICHOMONADIDAE. Although specimens are occasionally found in which more than three anterior flagella are present (reported in *T. augusta* by Samuels, 1949, and *T. foetus* in this paper) the significance of flagellar number as a taxonomic characteristic of generic value is not thereby destroyed. But even without regard to the number of anterior flagella, there are significant structural differences between *T. augusta* and *T. vaginalis* that seem to warrant generic distinction. On a morphological basis *T. foetus* belongs in the same genus as *T. augusta.* It is not now appropriate to use physiological characteristics of trichomonad flagellates in making generic distinctions. The name is therefore given in this article as *Tritrichomonas foetus* (Riedmüller, 1928) Wenrich and Emmerson, 1933.

OBSERVATIONS ON LIVING FLAGELLATES

Living culture flagellates were studied by dark-field illumination under paraffin-sealed coverslips, after they had become slowed down in their movements.

On all living specimens so observed, in which the anterior flagella could be counted with certainty, they numbered three and were usually almost equal to one another in length. For a short distance only they are united in a column, in which the separate flagella cannot be distinguished. The separated flagella appear fairly stout in dark field and are equal in thickness for all their length.

The flagella leave the cytosome on the dorsal side somewhat posterior to the narrow, rounded, anterior terminus (pl. 1, figs. 1–3; pl. 3, fig. 16). That origin is a constant feature which has been observed in many specimens. Most published drawings of *T. foetus,* based on fixed preparations instead of living flagellates, represent the origin of the flagella as apical (Wenrich and Emmerson, 1933; Witte, 1933; Wenrich, 1935; Cameron, 1938; Morisita, 1939; Morgan, 1944). There are, however, indications of the actual mode of origin in figures by Das Gupta (1936) and Morgan and Noland (1943). Riedmüller (1928) stated that the free flagella usually originate in a small indentation. The anterior protuberance of the cytosome is not a papilla extending along the bases of the flagella and moving with them, as it is in many devescovinid flagellates.

The anterior flagella are moved rapidly and somewhat irregularly. The pattern of movement described below was studied in specimens slowed under the coverslip until the body did not change position and the flagella could be followed. Beginning

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EXPLANATION OF PLATES

All figures are of *Tritrichomonas foetus* (Riedmüller). Technique, unless stated otherwise, Hollande cupric picro-formol fixative, Bodian protargol impregnation.

PLATE 1

FIG. 1. Drawing made from living specimen observed by dark-field illumination, × 4200.

FIG. 2. Photograph showing the parabasal body and the place of origin of the group of anterior flagella.

FIG. 3. Photograph showing the anterior flagella, posterior free flagellum, and impregnated projecting end of the axostyle.

PLATE 1

at the forward position, as represented in pl. 1, fig. 1, the flagella are moved separately back against the ventral side of the body. After they have all become posterior in direction, the group is drawn dorsally and then thrown rapidly forward. The most vigorous action appears to be exerted by the basal parts of the flagella, but the whole flagellum is capable of participating in active movement. The anterior parts may become curled while the rest is straight. When in the course of backward movement the flagellum strikes a small obstruction, the anterior part may curl as far as possible around the obstruction. The activity of the anterior flagella of *T. foetus* is similar to that of the group of anterior flagella of *Pentatrichomonas hominis* as described by Kirby (1943).

The undulating membrane has from three to five waves (pl. 2, figs. 6, 9, 10; pl. 3, fig. 14) and its margin shows two parallel filaments as far as the end of the region of attachment to the body. These features were reported in stained material by Wenrich and Emmerson (1933) and Das Gupta (1936). Sometimes the undulating membrane does not extend for the full length of the body. In larger specimens it often occupies not more than 2/3 or 3/4 of the length.

Extending beyond the end of the membrane is the long free posterior flagellum (pl. 1, figs. 1, 3; pl. 3, figs. 14, 16), which in both length and thickness is comparable to an anterior flagellum. The posterior flagellum is prolonged in a fine filament several microns in length. The undulatory movement of the membrane does not continue in the flagellum. Instead the flagellum beats forward and backward, and in one position it extends almost straight posteriorly.

When flagellates are kept in the space under a sealed coverslip they eventually die. Counts of flagella and study of details of movement can be made only on flagellates which are becoming slower in their activity and will be dead in a few hours. It cannot be certain that the pattern of activity is the same as when the flagellates are under favorable environmental conditions. Certain phenomena have been seen in some specimens which clearly are abnormal, and result from these unfavorable conditions.

Occasionally the undulating membrane is buried in the cytoplasm. It was observed in one specimen to undulate slowly, causing movements in the cytoplasm, while the free posterior flagellum lashed to and fro. A wavelike movement of the surface of the body results from the activity of a buried membrane.

Displacement of structures in *Tritrichomonas foetus* was described by me in 1947. The undulating membrane together with the costa was observed in one specimen to migrate five times down one side of the body and back up the other. In other specimens, anterior flagella were observed to depart separately from their original position and move into various positions at the surface of the body.

Dark-field illumination demonstrates a number of features of internal structure (pl. 1, fig. 1). The costa appears as a bright rod, tapering toward either end. The displacement of the undulating membrane described above indicates the close association between the membrane and costa, which is displaced with it. The edges of the axostyle appear as bright lines, and the axostyle projects in a pointed cusp from the posterior end. There is no evidence that the cusp is covered by cytoplasm. Toward the other end the axostyle is gradually expanded into a capitulum in the manner described by Wenrich and Emmerson (1933).

In all of the cytoplasm posterior to the nucleus on the dorsal side and for the

entire length on the ventral side many small granules were seen in living flagellates observed in dark field. The granules were in active Brownian movement, which was as vigorous when preparations were first made as several hours later. In fact, some flagellates that were moribund had granules in less active Brownian movement than did more vigorous ones. It appears that the cytoplasm was highly fluid in the flagellates so prepared for study.

A number of larger spherules, which appeared as rings in dark field, were present in the flagellates.

Twenty-one living specimens observed by dark-field illumination had a length of 14.8–22.6 μ, a width of 4.7–10.1 μ, and medians of 17.9 μ and 7 μ. The length given does not include the length of the projection of the axostyle, which ranged from 1.5 μ to 4 μ. The anterior flagella had a length of 11–17 μ, and the free posterior flagellum often about 16 μ.

OBSERVATIONS ON STAINED PREPARATIONS

The fixed specimens studied ranged down to a much smaller size than that of the living ones recorded above, the minimum being 9 $\mu \times 3$ μ, and Wenrich and Emmerson (1933) recorded specimens as short as 10 μ. As was noted by Witte (1933) some broader and even round forms occur in older cultures. Dimensions of some of the almost globular forms on my slides were about 13 $\mu \times 15$ μ.

In my iron haematoxylin preparations the flagella originated in what appeared to be a compact granule situated close to but not at the anterior extremity. Wenrich and Emmerson found the granule complex at the base of the flagella to appear commonly as a single body, but sometimes to consist apparently of a few closely clumped granules. Witte (1933) showed three distinctly separate granules in each of his three figures, which represented bodies with a single mastigont, two mastigonts, and multiple mastigonts. In fact, the granules he represented are so far separated from one another that if they were included in a single stained matrix to give the appearance often presented of a single large granule, that body would be extraordinarily large. Other studies do not corroborate this report of distinct and regular isolation of granules. In some of my specimens, however, some elements of the group were separated from others, and when an individual flagellum migrates in the body it seems to carry along a kinetosome. The seemingly single large granule of many specimens of *Tritrichomonas foetus* is probably a complex of several individual kinetosomes.

Silver-protein preparations show the flagella, undulating membrane, axostyle, and parabasal body with diagrammatic clearness. The costa was impregnated in some specimens (pl. 2, fig. 10) and not in others. The kinetosome complex was not impregnated. Frequent failure of that structure to give a positive reaction in this technique was reported by Dashu Nie (1950) in *Monocercomonas caviae*, *Hexamastix caviae*, and other flagellates of the guinea pig; and by Wenrich and Saxe (1950) in *Trichomonas micrôti* of rodents.

The base of the group of anterior flagella is a single, stout, deeply impregnated column (pl. 1, figs. 2, 3) ending bluntly in proximity to the region where the unimpregnated kinetosome complex lies. The distal end of each anterior flagellum may appear quite black when the rest of the flagellum is pale; and except when impregnation is very heavy it can be separately recognized as a granule or short rod (pl. 3,

fig. 21). In *Tritrichomonas foetus* it usually appears as a short, blunt rod which either lies in the same direction as the following part of the flagellum or is bent at an angle to it. A comparable terminal differentiation has been reported in other flagellates of the families MONOCERCOMONADIDAE and TRICHOMONADIDAE by Kirby (1945), Kozloff (1945), Honigberg (1947), Kirby and Honigberg (1949), Dashu Nie (1950), Wenrich and Saxe (1950), and Kirby and Honigberg (1950). It seems in the species I have studied not to be a swelling or knob, as it was reported by Nie (1950) to be in *Monocercomonas caviae* and *Hexamastix caviae;* but rather as shown in a diagram of the flagella of *Pentatrichomonas hominis* (Kirby, 1945, pl. 2, fig. 15) to be no thicker than the other parts, if as thick. In the dark-field studies I have made, no differentiation or enlargement has been seen. If there were a swelling, it would be expected that it could be seen in dark field.

The outer margin of the undulating membrane impregnates as a stout filament, somewhat but not greatly thicker than an anterior flagellum (pl. 1, fig. 3; pl. 3, fig. 14). It originates at the posterodorsal part of the kinetosome complex, quite separately from the group of anterior flagella. It continues without a break as the posterior free flagellum, which as in many related flagellates terminates in a filament. The marginal filament, like the flagella, appears to be much stouter in silver preparations than in haematoxylin-stained preparations. The two filaments shown by Wenrich and Emmerson (1933) at the membrane margin are close enough together so that they could be regarded as constituting the two edges of the marginal flagellum. Dark field also seems to show these two edges, and the outermost filament as seen by that technique is much more slender than the free posterior flagellum. For permanent preparations, iron haematoxylin is more dependable in demonstration of this detail of structure. It was observed in *Trichomonas guttula,* however, that occasionally silver preparations show two finer filaments instead of the stout structure (Kirby and Honigberg, 1950). If there is a single marginal element, which continues in the free posterior flagellum, and whose edges or whole substance may be revealed according to the technique used, the question as to which filament continues into the flagellum does not arise.

Silver preparations of *Tritrichomonas foetus* often show a slender filament paralleling the stout marginal flagellum and separated from it by a narrow clear zone, different in appearance from the grayish membrane substance between the fine filament and the body. It seems as though in these prepared flagellates the membrane substance was not in actual contact with the marginal flagellum in all places, or that there was some intervening differentiation.

PLATE 2

Photographs

FIGS. 4, 5. Iron-haematoxylin-stained specimens; in fig. 5, the ventral border of the broadened anterior part of the axostyle can be seen.

FIGS. 6, 7. Undulations of the membrane and loop form of the silver-impregnated parabasal body are shown.

FIG. 8. Greatly enlarged photograph of parabasal body in the loop form.

FIG. 9. Border of undulating membrane from dorsal aspect.

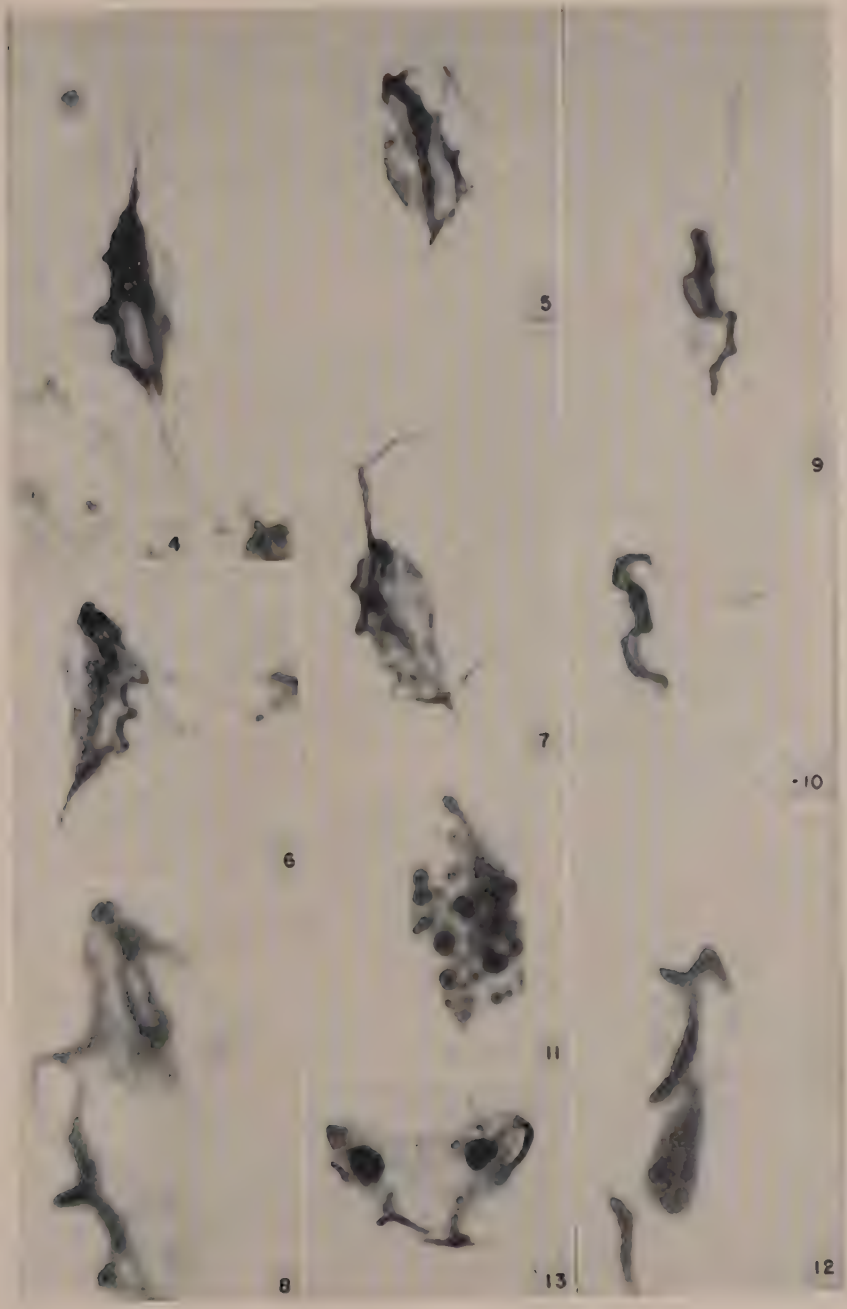
FIG. 10. Border of membrane from dorsal aspect and costa.

FIG. 11. Iron-haematoxylin-stained spherules, which occur in most specimens in bacteria-free culture.

FIG. 12. Greatly enlarged photograph of the sausage-formed parabasal body.

FIG. 13. Dimastigont specimen, in which the mastigonts are turning from the position of similar orientation to one of opposite orientations.

PLATE 2



Beginning at the anterior end of the flagellate and passing posteriorly on the ventral side is a narrow, lunate structure about a micron in length (pl. 3, figs. 17, 19). It impregnates deeply and is constant in occurrence and shape. Its position is on the capitulum of the axostyle. In *Tritrichomonas foetus* there is no pelta extending from the capitulum of the axostyle as in *Pentatrichomonas hominis.*

The capitulum and trunk of the axostyle, including the anterior enlarged capitulum (pl. 2, fig. 5; pl. 3, fig. 15), short projecting part (pl. 1, figs. 1, 3), and the chromatic ring at the place of emergence, appeared in my preparations exactly as described by Wenrich and Emmerson (1933).

The parabasal body of *Tritrichomonas foetus* was first found by Wenrich and Emmerson in preparations fixed in chrom-acetic. They described it as a short cylindrical or club-shaped structure lying between the nucleus and the costa. In my silver preparations it was usually demonstrated consistently and clearly. I did not find it to lie exactly between the nucleus and costa in any specimen. Instead it lies somewhat to the right of the nucleus. Its length is about 3–4 μ. Beginning at the kinetosome complex a large part of the parabasal body lies anterior to the nucleus, which is often about 3 μ posterior to the extremity of the body (pl. 2, fig. 12; pl. 3, fig. 16). The parabasal body approaches the anterior end of the nucleus and upon reaching it often turns to the right and dorsally (pl. 3, figs. 16, 20g). Here it sometimes terminates in contact with the nuclear membrane, sometimes continues free into the cytoplasm. The part of the parabasal body that lies beside the nucleus may be as much as half its length (pl. 3, fig. 20f), or at the other extreme only a small part touches the nucleus (pl. 3, fig. 20b, d).

In one series of preparations the parabasal body usually appeared as a solid-looking, sausage-shaped structure, sometimes straight but more often curved, and rounded at both ends (pl. 1, fig. 2; pl. 2, fig. 12). Sometimes it was not impregnated at all, though that was unusual with the lot of protargol used. In another series the parabasal body was impregnated only at the edge, and appeared as an elongated loop (pl. 2, figs. 6–8; pl. 3, fig. 20a–f). The shape of the impregnated element often seems to be like a link of a chain rather than a shell of which an optical section has a looplike appearance. When a parabasal body appears as a rod, focussing at a different level will often reveal the other edge of the loop. A ringlike or looplike shape of the part of the parabasal body impregnated under certain conditions has been reported in other trichomonad and hypermastigote flagellates (Kirby, 1944, species of *Trichonympha;* Kirby, 1949, *Foaina nana*).

The number of anterior flagella is characteristically three. Wenrich and Emmerson (1933) did not record any departures from this number in nondividing flagellates; and Witte (1933) wrote that the number of flagella is constant, variations not having been observed. A fourth anterior flagellum has been reported infrequently. Gehring and Murray (1933) and Morisita (1939) published photographs of specimens with four anterior flagella, and the latter author stated that Futamura in 1936 described a similar instance of an extra flagellum.

In silver preparations of flagellates from cultures I have observed several sorts of differences from the normal number and arrangement of flagella. One specimen had only two anterior flagella. Of four nondividing specimens with four anterior flagella, one had them grouped together, forming a single basal column; two had three flagella in one group and one somewhat separated and closely associated with

the place or origin of the undulating membrane (pl. 3, fig. 17); and one had the flagella in two groups of two, isolated at the base. In two dividing specimens out of a very large number observed, four anterior flagella were present in one of the mastigonts and three in the other (pl. 4, fig. 27).

As shown by these dividing specimens, the extra flagellum develops in the period of morphogenetic differentiation. Ordinarily the group of three parent anterior flagella separates into a single flagellum and a pair. In the mastigont retaining one parent flagellum, two new ones grow, and one new one develops in the other. Some factor determines that the outgrowing number shall be that necessary to complete the normal complement, and normally no more. An alteration in this morphogenetic influence, which would be a developmental departure from normal, probably inherent in the kinetosome complex, could result in outgrowth of two flagella in each mastigont. Then one individual would have four anterior flagella and the other three, just as has been observed. Recognition of this possibility, however, it not in conflict with the opinion that the genetically determined number of flagella is a valuable taxonomic characteristic.

Specimens have been found in which no part of the normal process of division had taken place, but which had five or six anterior flagella. The absence of the normal division process was shown by the presence of one nucleus in the interkinetic state, one undulating membrane and costa, and usually only one parabasal body. All these specimens had the usual group of three anterior flagella. In each of three flagellates, two additional flagella were present, separated from the group of three, and either close to one another (pl. 4, fig. 25) or isolated (pl. 3, fig. 18). In the third, two long flagella were present in the customary position, and with them a short flagellum; another long flagellum accompanied by an outgrowing new one was displaced to the ventral side of the body. Another specimen, in which there was no duplication of any other structure, had two groups of three flagella each.

Doubling of the axostyle is fairly frequent in the culture flagellates. The two axostyles lie side by side, the two cusps projecting from the posterior end. Sometimes in the same specimens the nucleus is broad and flattened. That the doubling of the axostyle is not a part of a normal division process is shown by the usual presence of only one set of anterior flagella, one parabasal body, and one costa and undulating membrane. A specimen with a double axostyle, two extra flagella, and a parabasal body with an abnormal angled shape is shown in pl. 4, fig. 25.

Several specimens were found in which, in the absence of any other doubled structures, there were two parabasal bodies.

The flagellates described above show that a variety of irregularities in morphogenesis, in which no orderly sequence has been traced, may occur in flagellates in cultures. These have not been described in *Tritrichomonas foetus* from its natural environment, in which thorough studies of division and morphogenesis are yet to be made. The irregularities may possibly occur under natural conditions, but it is more likely that the circumstances of cultivation are especially conducive to such happenings. Samuels (1949) found that morphological variations are common in trichomonads of amphibians in cultures. He reported axostyle duplication, large amoeboid nuclei, and occasionally an extra anterior flagellum. The extra flagellum may be inserted in the normal kinetosome complex, or may be attached to a separate granule well removed from this.

The irregularities described above can be found in appreciable number by examining a large number of preparations, but at the same time a much greater number of normal division stages will be seen. The division process is like that in other trichomonads: outgrowth of a new parabasal body, costa, and undulating membrane; discarding of the old axostyle and growth of two new ones (pl. 4, fig. 22); distribution of anterior flagella and outgrowth of one or two new ones to complete the set in each mastigont.

Gehring and Murray (1933) stated that the axostyle seems to split into two parts, and they found no evidence to support the opinion of Witte (1933) that the old axostyle is resorbed. This was a supposition by Witte based on interpretation of two-mastigont specimens, but he was correct. I have seen many specimens showing the process of resorption and outgrowth in *Tritrichomonas foetus*, and a large number of studies show that axostyles in trichomonads always originate in that manner (Kirby, 1944).

A great many specimens were examined in the effort to learn whether the whole original parabasal body is retained in one mastigont, or is discarded in part or completely. Unfortunately it was not possible to find out. The last method, complete discard, is improbable. In spite of the fact that a large number of division stages were seen, no detached parabasal substance was found in the cytoplasm of any specimen. The deep impregnation and striking contrast of parabasal substance in silver preparations would have made it possible to detect such detached substance readily. The critical stage, in which a small new parabasal was growing out for one mastigont, was not found.

Provision of a set of three anterior flagella for each mastigont is completed before division of the body (pl. 4, figs. 22–24). Consequently practically all solitary flagellates have three anterior flagella, except for the occasional abnormalities in morphogenesis that have been described.

The greater number of late division stages, in which there are two mastigonts, belong to one or the other of two general types. In one, the two groups of anterior flagella and the nuclei are close together and the axostyles are parallel (pl. 4, figs. 22, 27). The two undulating membranes are roughly parallel and lie either in the direction of the longitudinal axis of the body or curved spirally about it. The two membranes may be close together or on opposite sides of the body. In the other type, the cytoplasmic body has about the same size and shape but the mastigonts lie in opposite directions (pl. 4, figs. 23, 24). The anterior flagella are at opposite ends

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PLATE 3

All figures except 21 × 3365

FIG. 14. Undulating membrane from dorsal aspect and posterior free flagellum ending in a fine filament. Compare the specimen in photograph, figure 10.

FIG. 15. Axostyle with expanded anterior part. Parabasal body.

FIG. 16. Entire specimen showing the nucleus and the mastigont organelles demonstrated by silver impregnation. Typical relationship of parabasal body to nucleus.

FIG. 17. Specimen with a fourth flagellum, showing also the silver-impregnated small lunate structure on the anteroventral end of the axostyle.

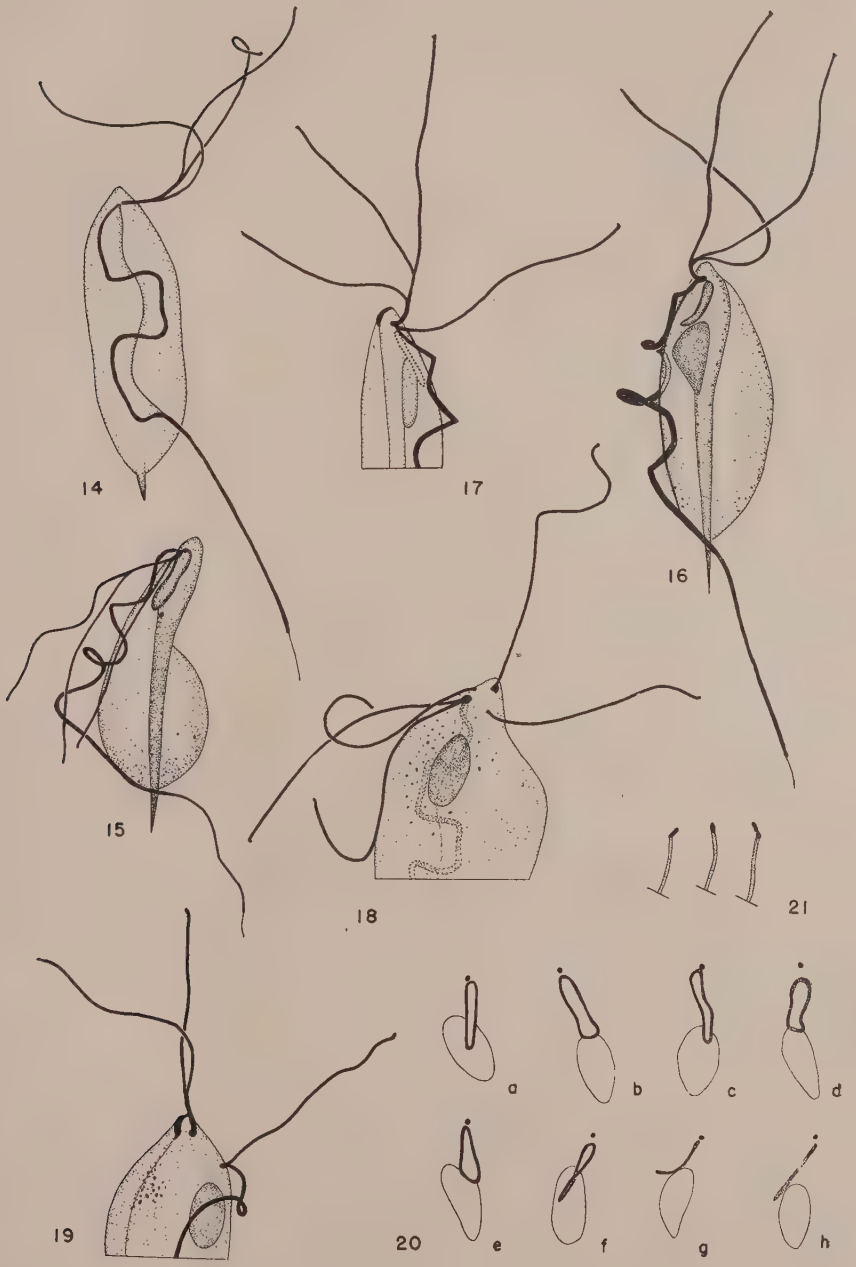
FIG. 18. Two extra anterior flagella, separate origins of flagella.

FIG. 19. One of the flagella and the undulating membrane removed from the normal position.

FIG. 20. Forms of the parabasal body: *f* is seen partly on edge; *g* and *h* are edge views of the loop-shaped structure.

FIG. 21. Diagrams showing the terminal differentiation of the anterior flagella.

PLATE 3



of the body. The undulating membranes run along the sides of the body, and at first each reaches to the opposite end (pl. 4, fig. 24). The axostyles parallel one another in opposite directions, the posterior end of each being near the anterior end of the other.

A few specimens have been found in which the mastigonts had been migrating, shifting from the original orientation in the same direction to a position in which they lie in opposite directions (pl. 2, fig. 13). This is accomplished by a turning of one or both within the cytosome, which may be caused to bulge, but there is no deepening furrow. It is this turning that corresponds to the longitudinal division characteristic of flagellates. Later the two mastigonts draw apart in opposite directions, the cytoplasm being drawn out until it constricts and separates in the middle part, at the time when the mastigonts are oriented end to end. If the shifting in direction involves only one mastigont, and that cannot be affirmed or denied, the cytosome actually divides transversely to its original axis. Nevertheless, there is obviously no justification for comparing the division of the trichomonad with transverse division of ciliates. The original orientation of the mastigonts following nuclear division shows that no such comparison can logically be made. Honigberg (MS) observed the migration and separation of mastigonts in living *Trichomonas prowazeki,* and he pointed out that division is a modification of the longitudinal type, as it is in devescovinid flagellates (Kirby, 1944).

Multimastigont forms sometimes occur in cultures of trichomonad flagellates, and have been reported in various species. They are sometimes, though less often, found under natural conditions. In a survey of the preparations of *Tritrichomonas foetus* nine specimens were found with three mastigonts and nine with four. Gehring and Murray (1933) published a photograph of one with four. Witte (1933) reported exceptionally finding multiple division forms on blood-agar plates, and he figured one with four mastigonts. It is possible that multimastigont forms may have originated from multipolar division figures. Cleveland (1938) reported numerous instances of tripolar and quadripolar achromatic figures in the hypermastigote, *Barbulanympha.* Kirby (1946) found a quadripolar figure in the flagellate form of the trichomonad *Gigantomonas herculea.* It is more likely, however, that origin by successive normal divisions accounts for the multinucleate forms. The forms with three mastigonts would then have resulted from detachment of one, or division of only one of the original two nuclei.

Kofoid and Swezy (1915) found multimastigont forms to be common in *Tritrichomonas augusta.* They recorded and figured an interesting series of observations on a form which when first seen had five sets of organelles and five nuclei. Indi-

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PLATE 4

All figures × 3365.

FIG. 22. Specimen with two mastigonts fully developed except for the axostyles, two of which are growing out, while the projecting end of the discarded axostyle is still present.

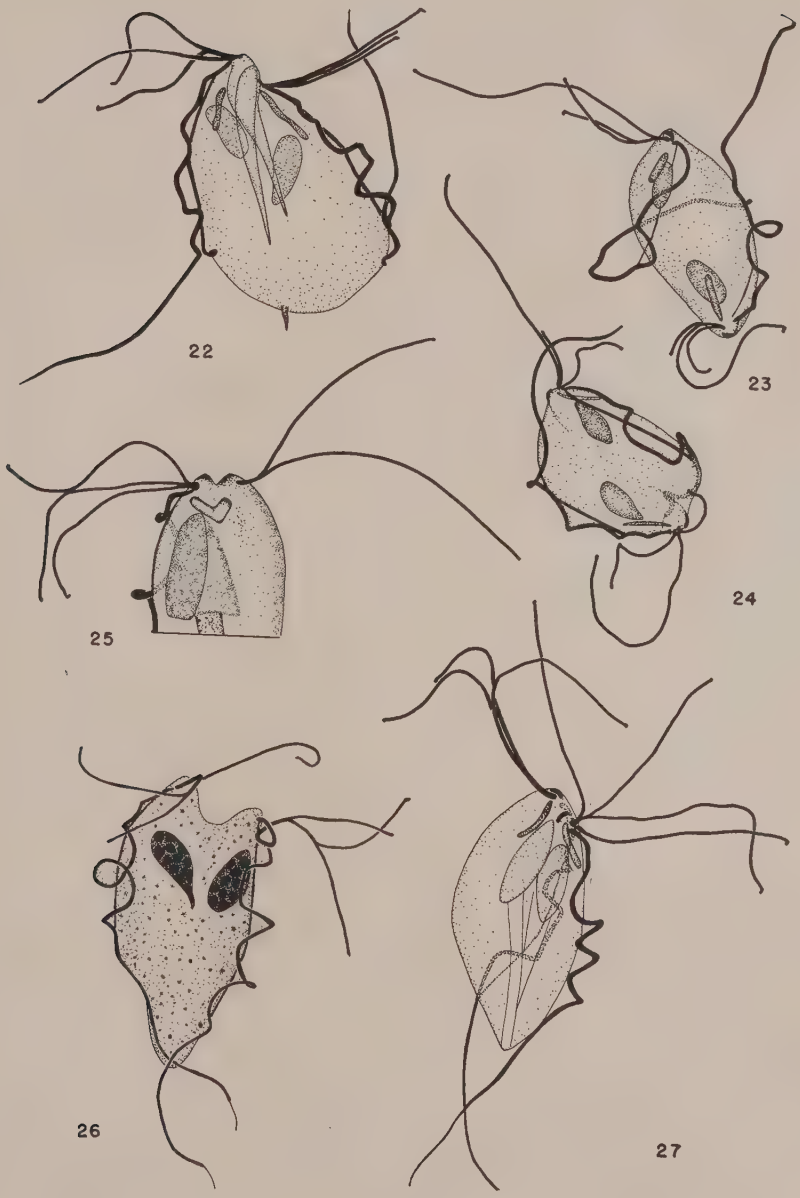
FIG. 23, 24. Specimens in which the mastigonts have turned in opposite orientations. The lunate structure is present at the anterior end of each axostyle.

FIG. 25. Abnormal flagellate in which the nucleus is enlarged, the parabasal body is bent, and there is a group of two anterior flagella separated from the normal set of three.

FIG. 26. Anterior longitudinal furrow as though in beginning fission. Instead of deepening to separation this will later be obliterated, as the mastigonts come to lie in opposite directions. Compare photograph, figure 13.

Fig. 27. Double specimen with two fully developed mastigonts.

PLATE 4



viduals drew out and detached themselves from this body, until all were isolated. Kofoid and Swezy used terminology adopted from that of the coccidian life cycle, writing of multiple fission and merozoites. Kofoid (1941) considered the life cycle of *T. augusta* to involve alternating periods of binary and multiple fission, and included both types of fission in a life-cycle diagram. It is more likely, however, that the multimastigont forms in trichomonads represent a departure from the normal course of events, and are no more properly to be regarded as part of the life cycle than are the multicomposite ciliates described by Fauré-Fremiet (1945). Fauré-Fremiet stated that various causes, accidental or experimental, may disturb the mechanism of division and arrest the separation of tomites, resulting in double or multiple ciliates.

SUMMARY

Studies of *Tritrichomonas foetus* were made from living material, observed especially by dark-field illumination, and from preparations, especially those made by silver impregnation technique. The observations add to previous knowledge of the flagellate certain details about the origin, grouping, and activity of the anterior flagella; the position, form, and relationship to the nucleus of the parabasal body; and the morphology and activity of the undulating membrane and the free posterior flagellum. The parabasal body appears solid in some silver preparations and has the form of an elongated ring in others. As in many other trichomonads, the posterior flagellum is an acroneme type of flagellum. Some of the flagellates in culture show the results of morphogenetic abnormalities, having one or two extra anterior flagella, enlarged and misshapen nuclei, duplicated axostyles, and arrested cytoplasmic division. In a greater number of others the normal course of events in division may be studied. The outgrowth of new anterior flagella, giving two sets of three, is completed before cytosomal division. The two mastigonts, at first parallel and both directed anteriorly, come to lie in opposite directions and draw apart. This is essentially longitudinal division, although the body is not furrowed longitudinally as in the typical form of that division.

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TWO NEW SPECIES OF MACRONYSSIDAE, WITH NOTES ON SOME ESTABLISHED GENERA (ACARINA)

R. W. Strandtmann[1] and O. E. Hunt[2]

We have had the pleasure and privilege of examining recent and rather extensive collections of mites taken from small mammals. These were collected in Georgia by H. B. Morlan and in Texas by W. F. Blair, mammalogist with the University of Texas, and R. B. Eads and G. C. Menzies, entomologists with the Texas State Department of Health. To these scientists we wish to extend our appreciation for the opportunity to study these mites.

In the collection was a large series of a new species found on skunks, *Mephitis* spp. and *Spilogale* spp., in Georgia, Oklahoma, and Texas.

Hirstionyssus staffordi n. sp.

Figs. 1–15

A very distinctive mite averaging slightly over 0.5 millimeter in length. The female has only two pairs of setae on the sternal plate, and the dorsal plate has a characteristic narrow, blunt posterior projection. The male has extremely short setae on the holoventral plate.

Female. (Figs. 1–7) : Gnathosoma: Arms of the chelae are relatively short; chelicerae are slender, as shown in Figure 6. The epistome (Fig. 4) apparently has a smooth acute anterior margin, reaching to the end of the fourth segment of the palpus. Hypostome (Fig. 7) has a single row of teeth. The details of the gnathosoma are difficult to distinguish; see Figure 7 for size and position of the four pairs of setae. The middle two pairs of setae are very small and close together.

Dorsal aspect. Dorsal shield (Fig. 2) covers most of the dorsum. There is always a complete ring of soft, striated skin surrounding the dorsal shield, with about 13 pairs of fairly heavy setae; shield is slightly concave behind the pronounced shoulders, coming to an abrupt point posteriorly. There are about 23 pairs of setae and numerous pores on the dorsal plate. Peripheral setae are longer and heavier than the median short weak setae.

Ventral aspect. The sternal plate (Figs. 1, 5) has two pairs of setae: the first pair is submedian on the edge of the anterior margin; the second pair is lateral, opposite the middle of coxa II. Another pair appears to be in the soft body without sclerotization. The sternal plate is about 3.5 to 4 times as wide as long. Anterior margin is slightly curved; posterior margin is almost straight, but corners appear to be slightly eroded. Setae are large and long, tapering to a slender point. The two pairs of setae on the plate and the pair off the plate are about the same size. There are three small sclerotized plates situated between coxae II and III, III and IV, and opposite IV, as shown in Figure 5. Genitoventral plate has a wide, rounded posterior end with a pair of setae opposite coxa IV. Anal plate is oval; anus is in the anterior half; a pair of setae is on level with the anterior margin of the anus. Postanal seta is about the same size as paired setae. A well-developed cribrum is on the posterior part of the plate. All the body plates have no markings, or only a few very weakly developed lines. The ventral area is heavily striated, with about 13 pairs of setae. The posterior end of the body is sometimes bilobed. The stigma and peritreme are entirely opposite coxa III; peritreme is wide and short.

Legs. All legs have claws; lengths of legs: I—344 μ, II—280 μ, III—280 μ, IV—344 μ. Legs I and II are somewhat heavier than legs III and IV. Legs I and II dorsally have two long setae on the femur and one on the genu. Tarsus II has a pair of curved clawlike spines ventrally near apex (Fig. 3). Coxa I has a blunt spur and two setae. Coxa II has two spurs; the inner spur is bifid (or appears to be in some mounts) ; the marginal one is blunt with a seta at the base. The usual antero-dorsal spur is wide at the base and tapers to a point. Coxa III has two spurs; the inner spur is bifid (or appears to be in some mounts). Coxa IV has a spur at the base (Fig. 5). Length of body, not including the gnathosoma, 550 μ; width 330 μ.

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Male. (Figs. 10–14) : Gnathosoma : Arms of the chelae similar to those of female, except heavier and stronger (Fig. 11). Epistome appears same as the female. The hypostome has a single row of teeth. It is difficult to make out all the structures of the gnathosoma, but the arrangement of the setae is the same as in the female, and is shown in Figure 13. The tritosternum has a very pale outer margin; toward the end may be seen a number of very fine indistinct hairs.

Dorsal aspect. Dorsal shield (Fig. 10) is like that of the female except that it covers a little more of the dorsum and has a less pronounced posterior projection. There are about 23 pairs of setae and several pairs of pores. Peripheral setae are all heavier than the medial short setae.

Ventral aspect. The holoventral plate (Fig. 12) is broadest at the anterior end; corners project between coxae I and II, II and III, and III and IV. There are about 8 pairs of very short setae on the plate, the first pair being very tiny. The anal plate is separated by a suture; anal setae are longer than the others on the plate; posterior end of the anal plate has a well-developed cribrum. The soft portion of the ventral area is heavily striated and has about 9 pairs of strong setae. The stigma and peritreme are same as in the female. The legs are very similar to those of the female, but a little heavier, and the hind legs are longest. Lengths of legs: I—293 μ, II—250 μ, III—250 μ, IV—320 μ. The setae are a little heavier than those on the female. There are four pairs of fairly heavy ventral setae on tarsi III and IV. Tarsus II (Fig. 14) has a pair of long curved clawlike spines at the apex. The spurs on coxae I and II do not always show up clearly. The bifid spur on coxae II and III is sharp-pointed on one corner. Length of body, not including the gnathosoma, 510 μ; width 344 μ.

Deutonymph: (Figs. 8, 9) : The gnathosoma, including the chelicerae and epistome appear similar to those of the female. Dorsal plate (Fig. 9) covers only part of the dorsum; peripheral setae are all heavy; middle setae are small and weak. A pair of long setae are at the posterior end. Sternal plate (Fig. 8), which ends about the middle of the fourth coxa, has four pairs of setae. Coxa I has a large spur; coxae II and III each has an indistinct marginal spur and a bifid inner spur; and coxa IV has one small spur. Tarsus II has two small indistinct terminal clawlike spurs. The stigma and peritreme are short and thick and entirely opposite coxa III. The body is heavily striated, and has about 12 pairs of normal setae behind and below the fourth coxae near the anal plate. The anal plate is somewhat pear-shaped, with the usual three setae; the posterior end of the anal plate has a distinct cribrum. Length, 350 μ; width 225 μ.

Protonymph: We have seen no free protonymphs, but we did find two females each containing a protonymph (Fig. 15). The details of structure and setation are impossible to make out. Apparently there is a stigma but no peritreme between coxae III and IV.

Larva: Unknown; perhaps always absent?

Type host: Spilogale putorius (L.), the spotted skunk.

Type locality: Grady County, Ga.

Types: Holotype female and allotype male, mounted on separate slides, each labeled; GEORGIA, Grady County, Nov. 23, 1948, GM-902, H. B. Morlan, Collector. U. S. National Museum No. 1889.

Paratypes: ex S. putorius (L.); GEORGIA, Decatur County, Oct. 13–15, 1948, H. B. Morlan, collector, 11 females, 3 males, 1 deutonymph. Grady County, Oct. 12–Nov. 23, 1948, H. B. Morlan, collector, 18 females, 1 male. *ex Spilogale leucoparia* Merriam; TEXAS, Presidio County, July 22, 1947, W. F. Blair, collector, 2 females, 1 male, 1 deutonymph. *ex* "Civet Cat" *Spilogale interrupta* (Raf.); OKLAHOMA, Cushing, Oct. 28, 1938, K. C. Emerson, collector, 1 female. *ex Mephitis elongata* (Bangs); GEORGIA, Brooks County, March 15, 1946, and Dec. 14, 1948, H. B. Morlan, collector, 2 females, 4 males. Newton County, July 8, 1936, Travis and Komarek, collectors, 6 females, 2 males. Grady County, Jan. 21, 1949, H. B. Morlan, collector, 1 female. Decatur County, Aug. 26, 1948, H. B. Morlan, collector, 2 females, 3 deutonymphs. *ex Mephitis mesomelas* Lichtenstein; TEXAS, Travis County, March 7, 1948, Miller, collector, 1 female, 1 male. Hays County, July 14, 1947, and March 7, 1948, Kuykendall Ranch, Eads and Menzies collectors, 9 females, 8 males.

This is a total of 53 females, 20 males, and 5 deutonymphs from two genera and five species of skunks. Paratype slides are at present in the collection of the National Museum, the Texas State Health Department, Austin, and in the collections of the authors.

Remarks. Hirstionyssus staffordi differs from all other species of the genus in having only two pairs of setae on the sternal plate of the female and in the extremely short setae on the holoventral plate of the male. In Fonseca's (1948) key to the

genera of the Macronyssidae this species runs to *Neoichoronyssus,* because of only two pairs of setae on the female sternal plate and the presence of spines on coxa I of both sexes. However, we do not attach as much importance to a reduction of the female sternal plate as does Fonseca, for reasons given later in this paper. If this species is analyzed in Fonseca's key as if three pairs of sternal setae were on the plate, it runs easily to *Hirstionyssus,* and we believe that is where it belongs.

We found remarkably little variation in the many specimens from the various hosts, except in size. Specimens from *Mephitis* seem to be consistently larger than those from *Spilogale,* although there is some overlapping in size range. Specimens from *Mephitis* average about 669 μ long, as compared with about 550 μ for those from *Spilogale.*

The mite is common on skunks, but only rarely is a host heavily infested. We have no records of it from any other animal. To date we have records only from Georgia and Texas. This gives it a known range in southern United States from near the Eastern Seaboard to the foothills of the Rocky Mountains in Trans-Pecos, Texas.

Perhaps the most interesting feature is the two females with protonymphs. The details of structure are almost impossible to make out but the presence of four pairs of legs is easily discerned and the stigmal openings on one specimen are readily seen. Figure 15 shows both cases. The sketches were made with the aid of the camera lucida. In both cases the nymph has the posterior end forward and the venter toward that of the female. Figure 15*b* shows the nymph with legs extended. This may be due entirely to pressure in mounting, but what appears to be a cast skin is seen at the anterior end of this nymph; also, the stigma is visible in this specimen and not in the other, suggesting that *b* had shed the larval skin within the mother and *a* probably had not.

One wonders whether giving birth to protonymphs is the normal procedure in this mite. This habit certainly is not so rare a phenomenon in mites as some acarologists seem to think (Turk, 1947). Radford (1937) has noted it in *Ophiopneumicola bedfordi* (Radford) (syn. *Entonyssus bedfordi* Radford) and Strandtmann (1946) has reported it for *Haemolaelaps glasgowi* (Ewing) (syn. *Atricholaelaps sigmodoni* Strandtmann). Since then we have seen it also in females of *Haemolaelaps megaventralis* (Strandtmann) and in a female of *Spinturnix iowae* Keegan. Turk (1947) states that this phenomenon has been occasionally reported for *Liponyssus* forms and that it occurs with certainty in a bat parasite, as reported by Vitzthum in 1931 (*vide* Turk, 1947).

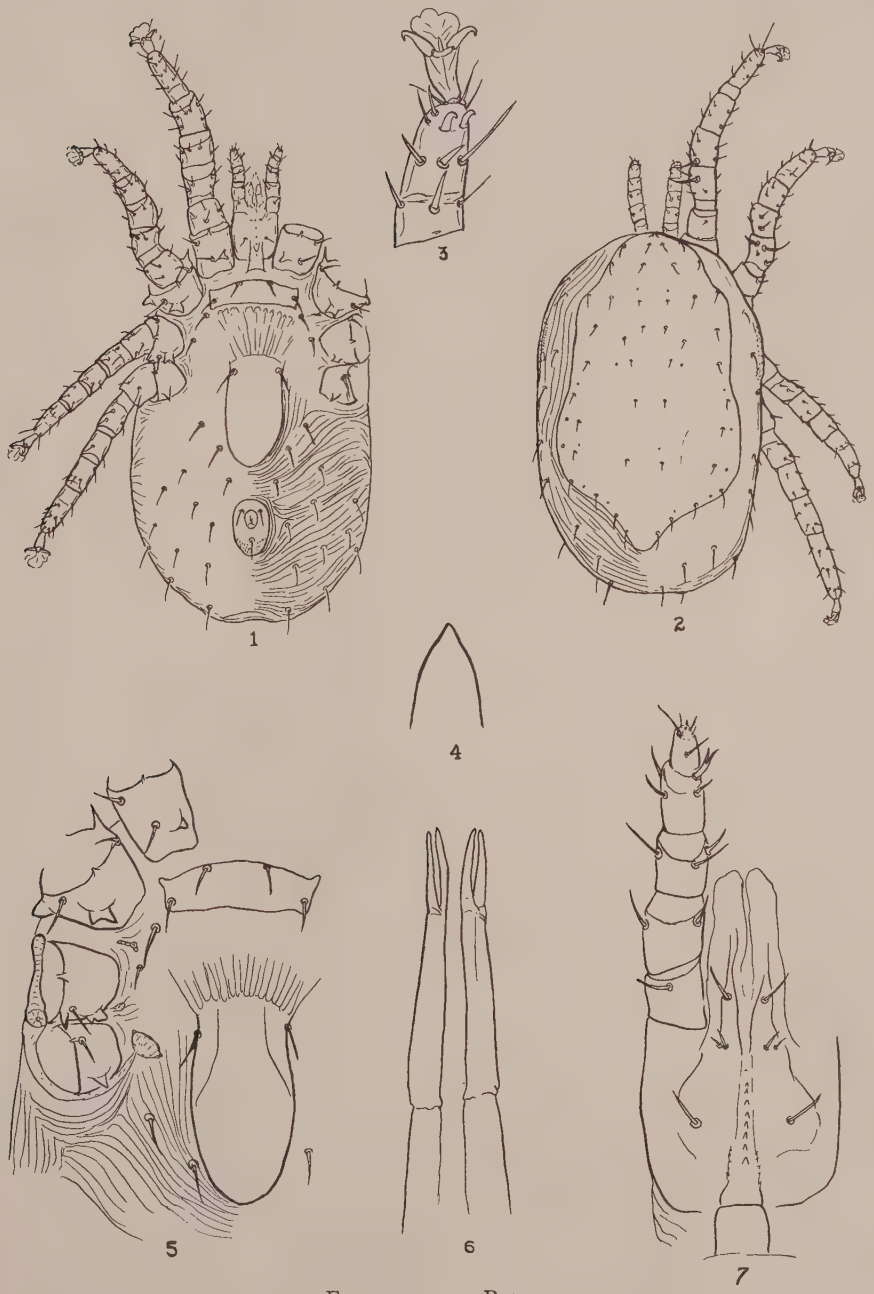
This mite has been named for Dr. E. W. Stafford, eminent professor, who has inspired many students in the study of entomology at Mississippi State College.

Ichoronyssus quadridentatus, n. sp.

Male. (Figs. 16–22): Gnathosoma: Arms of the chelae are short and stout; the movable arm bears a slightly curved spermatophore carrier; the chelicerae are stout (Fig. 17). The epistome is transparent and very difficult to distinguish, but apparently is rather short and acute, as in Figure 20. Hypostome has about nine contiguous rows of single teeth. A spur on the first joint of the palpus bears a tiny seta at its apex (Fig. 18). The corniculi apparently are very delicate and elongated. The lingula is covered in part with a pair of very transparent structures, as shown in the figure.

Dorsal aspect. The dorsal shield (Fig. 21) covers most of the dorsum. There is always a complete ring of soft striated skin, with a few long heavy setae surrounding the dorsal shield, and about seven pairs of very long slender setae at the posterior end. The shield is widest in

PLATE I



EXPLANATION OF PLATES

PLATE I. *Hirstionyssus staffordi* new species, female.

FIGS. 1 and 2, ventral and dorsal views; FIG. 3, tarsus II; FIG. 4, epistome; FIG. 5, enlarged view of coxae and ventral plates; FIG. 6, chelicerae; FIG. 7, ventral view of gnathosoma.

the shoulder regions and tapers evenly to a broad, heavily sclerotized, four-toothed plate at the posterior end. This plate has a pair of lateral setae, a pair of very short basal setae, and two pairs of big blunt thornlike spurs (Fig. 22). Anterior and peripheral setae of the dorsal plate are longer than the small mid-dorsal and posterior setae.

Ventral aspect. The holoventral shield (Fig. 16) is divided. The sternal-metasternal plate is widest at the posterior of coxa II and ends just back of coxa IV. This plate is sclerotized and has an irregular network of fine lines. There are five pairs of setae and three pairs of pores on this plate. Ventri-anal plate has about seven pairs of setae, including the paired anal but not the single post-anal. The post-anal seta is a little larger than the others on the plate. The plate is sclerotized and has an irregular network of fine lines. The ventral area is striated and has 20 to 24 setae on each side of the ventri-anal plate. The setae near the margin of the body are short and heavy, those near the plate are slender, and those on the posterior end are very long and slender. The stigma is opposite the anterior part of coxa IV. The peritrematalia curls around and behind coxa IV. The peritreme ends at the anterior margin of coxa II.

Legs. All legs have claws; lengths of legs: I—470 μ, II—395 μ, III—395 μ, IV—520 μ. The femora of legs I and II have an apical pair of slender setae; femur IV has one apical slender seta. Tarsus I has a group of what appears to be sensory hairs or setae dorsally (Fig. 19). Coxae II and III each have a small chitinous bump or elevation but there are no true spines, except the usual dorsal one on coxa II. Length of body not including the gnathosoma 535 μ.

Type: Male, Thomas County, Ga., April 8, 1947, H. B. Morlan, collector. U. S. National Museum No. 1890.

Type host: Eptesicus fuscus (Beauvois).

Type locality: Thomas County, Ga.

Paratypes: One male; Thomas County, Ga. February 26, 1948; H. B. Morlan. *ex Myotis lucifugus* (Lec.). The type and paratype slides are both in the National Museum. Female and immature stages unknown.

Remarks: The plate on the dorsum with the four dentates will separate this species from all other known species of this genus.

We have only the two males above mentioned. We have not seen any females or nymphs from *Eptesicus* that could conceivably belong to this species. We have seen many female mites from *Myotis,* but they are so similar to males of another species that they must be dismissed as a possibility of being the opposite sex of *I. quadridentatus.*

Remarks on Some Established Genera

One of the finest taxonomic papers on parasitic mites is that of Fonseca (1948). It shows a background of painstaking research and is a great step toward the final solution of the taxonomic muddle this group of mites has been in for so long. It is unfortunate that the name *Liponyssus* should have been so freely interpreted in the past and enjoyed such wide usage. A glance at Kolenati's (1859) drawings and descriptions should have been sufficient to convince any critical worker that his genotype of *Liponyssus* was completely different from the species that were constantly being added to the genus. Indeed others have called attention to it and the term Macronyssidae, as Fonseca points out, was originally proposed by Oudemans (1936). One might question the logic of retaining *Liponyssus* as a monotypic (and aberrant) genus without also retaining the family name Liponyssidae. However, one must agree with Fonseca that it is best to have as a type genus one that is known and also representative of the family. And *Macronyssus* is such a genus.

In spite of Fonseca's excellent and thorough treatment of the genera, there will be those who will disagree in some particulars, chiefly because of the unwillingness of all acarologists to place the same weight on certain characters. The present writers do not place as much emphasis on the reduction of the sternal plate in the female as does Fonseca. For instance, *Neoichoronyssus* is separated from allied

genera chiefly because it has only two pairs of setae on the sternal plate. But a study of Fonseca's original description of *Liponyssus wernecki* (1935) (the type and only species of *Neoichoronyssus* Fonseca, 1941) shows the third sternal setae so near the plate that they may as well be considered as being on it. We have seen many specimens of *Neoichoronyssus wernecki* (Fonseca) from *Didelphis* in the United States and find considerable variation in this character. Some unquestionably have the seta on the plate and others distinctly not. Some individuals have the third seta off the plate on one side and on it on the other. Hence we believe this is a variable character and of little taxonomic significance. However, we believe *Neoichoronyssus* is a good genus, but for characters other than those of the sternal plate. The holoventral plate of the male is narrow and undivided and the genitoventral plate of the female is pointed rather than rounded. This combination of characters is not true for its nearest allies, *Hirstionyssus* and *Ichoronyssus*.

The genus *Hirstionyssus* may be characterized as follows: Dorsal shield entire, tapering abruptly at the posterior end; coxae with ventral spines; holoventral plate of the male entire and slightly expanded.

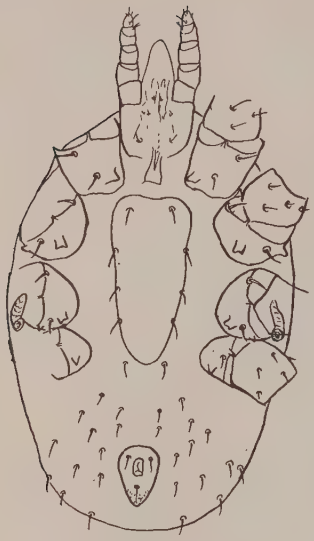
The genus *Hirstionyssus* is completely justified. Our experience has been, along with Jameson (1950) that species of *Hirstionyssus* are found on small mammals other than bats, and one wonders about the type being recorded originally from a bat. Perhaps it was accidental on the bats Kolenati had, or perhaps *Hirstionyssus arcuatus* is a universal parasite. Nevertheless our experience is that *Hirstionyssus* is not found on bats and *Ichoronyssus* only on bats.

In the *Ichoronyssus* complex we believe Fonseca has split hairs a little too fine. *Lepronyssus* Kolenati (1858) is retained because the genitoventral plate is scaly, but this is true also of species included in *Ichoronyssus*. The scaly appearance of the genitoventral plate is produced by the arrangement of the lines normally present on the shields of mesostigmatic mites and differs in the various species here considered not in presence or absence but only in degree of distinctness. In all respects (*vide* Hirst's drawings and descriptions, 1921) the species that Fonseca places in *Lepronyssus* agree with *Ichoronyssus,* including the fact that all are found on bats, and we believe *Lepronyssus* Kolenati should be considered a synonym of *Ichoronyssus* Kolenati.

Chiroptonyssus Augustson, 1945, also has very much in common with *Ichoronyssus*. The male has a divided ventral plate, the coxae have the slight, rounded elevation and it is found on bats. Also chelae of the male are very similar to other species (see Fig. 11). It differs, as Fonseca points out, in having long and slender fore legs and a reduced sternal plate in the female. The male differs further in that it has a very prominent spur on femur IV and less prominent ones on femora III and II, and in the configuration of lines on the ventri-anal plate. Ordinarily these lines form a reticulate pattern, but in *robustipes* (the type and only species of *Chiroptonyssus*) the lines are close, prominent, and transverse. However, we believe these are only specific characters, and therefore the name *Chiroptonyssus* Augustson, should be a synonym of *Ichoronyssus* Kolenati.

A genus not mentioned in Fonseca's paper, but which should be included in the Macronyssidae, is *Spinolaelaps* Radford 1940 (*Spinolaelaps jacksoni* Radford, monotypic). The genus was established primarily on the basis of an enlarged genitoventral plate of the female, bearing several pairs of setae. The male has a

PLATE II



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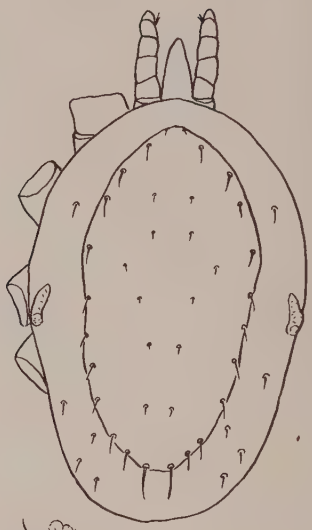

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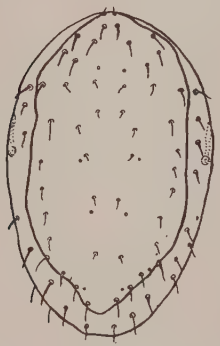

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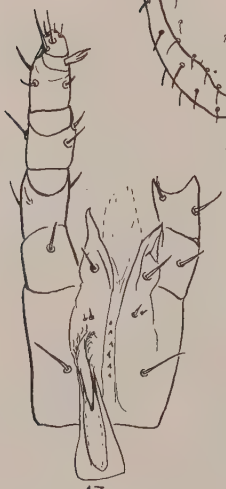

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divided ventral plate and the species is found on bats. A paratype female in the National Museum does not show the genitoventral plate as Radford has it illustrated. However, the specimen is excessively cleared and fails to show the critical characters distinctly, so we may be mistaken on this point. The chelae, however, are not laelaptine, and the genus should rightly belong in the MACRONYSSIDAE, very close to *Ichoronyssus* if not actually a synonym of it.

Leiognathus sylviarum Canestrini et Fanzago, 1877, should be mentioned. Fonseca established the validity of *Leiognathus* and states it has several striking generic characters. However in the generic diagnosis and in the key to genera there is nothing to distinguish it from *Bdellonyssus* Fonseca, 1941, except that the sternal plate of the female has only two pairs of setae. *L. sylviarum* is a very common species on the birds in the United States and we have seen many specimens. As in *Neoichoronyssus* the sternal plate is variable and many individuals have one or both of the third pair of sternal setae on the plate. Hence we believe that *Bdellonyssus* Fonseca and *Leiognathus* Canestrini et Fanzago are synonyms. However, Ewing (1947) pointed out that *Leiognathus** is preoccupied (by *Leiognathus* Lacepède 1802, a genus of fishes) so *Bdellonyssus,* being the next available name, is retained.

The above discussion is not to be construed as an adverse criticism of Dr. Fonseca's paper. A critical review of each genus will no doubt result in some synonyms and new combinations, but it is Dr. Fonseca's fine paper that will make this new classification possible. His monograph combines excellent illustrations with clear, concise descriptions which stress salient characters better than any previous work of a similar nature.

There has been some question as to the proper spelling of *Liponyssus.* Vitzthum (1932) pointed out that Kolenati introduced the name in 1858 as *Liponissus* and that this spelling therefore should be the correct form. Vitzthum indeed was consistent in using this spelling.

However Kolenati in 1859 used the spelling *Liponyssus* and called the mites of this genus by the common name "fatmites." Hence it seems evident that *Liponyssus* is derived from two two Greek words, *lipos* meaning fat and *nysson* meaning prick. At least this seems logical. Apparently *nissus* has no meaning. Also, all the other generic names proposed by Kolenati for mites of this group, such as *Ichoronyssus, Macronyssus,* etc. are written with a *y* so it seems that Kolenati meant to use the Greek word for prick throughout and in his 1858 paper the name was simply misspelled.

According to Art. 19 of the Rules of Zoological Nomenclature the original orthography of a name is to be preserved unless an error of transcription, a *lapsus*

* Since this was written, we have received Dr. Radford's paper, "Systematic Check list of Mite Genera and type species" (Union Internationale des sciences biologique. Serie C. No. 1, 1950). On page 223 of that paper Dr. Radford proposes the name *Fonsecaonyssus* for *Leiognathus* Canestrini, 1885, *non Leiognathus* Lacepède, 1802. *Fonsecaonyssus* must therefore, in our opinion, also be added to the synonymy.

PLATE II. *Hirstionyssus staffordi* new species, male and deutonymph.

FIGS. 8 and 9, ventral and dorsal views of deutonumph; FIG. 10, dorsum of male; FIG. 11, chelicera of male; FIG. 12, ventral view of male; FIG. 13, ventral view of gnathosoma and tritosternum, male; FIG. 14, ventral view of tip of tarsus II, male.

calami, or a typographical error is evident. It would seem that Kolenati considered a *lapsus calami* to have occurred in the original paper and considered *Liponyssus* as the correct orthography.

SUMMARY

Two new species are described: (1) *Hirstionyssus staffordi* from the skunks *Spilogale putorius* (L.), *S. leucoparia* Merriam, *S. interrupta* (Raf.), *Mephitis elongata* (Bangs), and *M. mesomelas* Lichtenstein, taken in Georgia, Oklahoma, and Texas, U. S. A.; male, female, deutonymph, and protonymph are described and

PLATE III

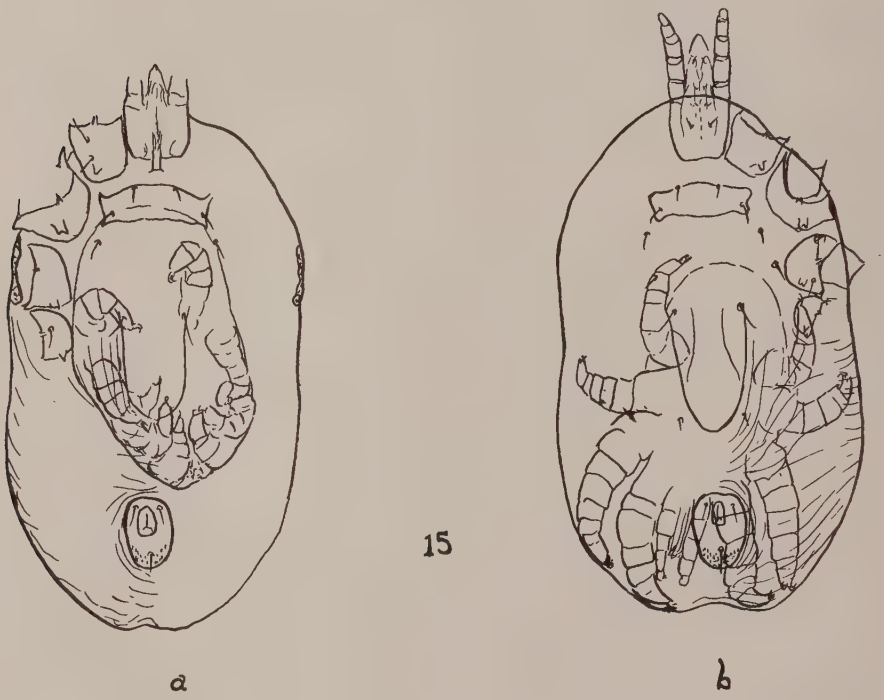

PLATE III. *Hirstionyssus staffordi* new species.
FIG. 15 a and b, showing protonymphs within the female.

figured. (2) *Ichoronyssus quadridentatus* from the bats *Eptesicus fuscus* (Beauvois), and *Myotis lucifugus* (Lec.), taken in Georgia, U. S. A. The male only is known.

Liponyssus Kolenati, 1858 (genotype, *L. setosus* Kolenati, 1858) is of unknown identity and therefore should not be used as the typical genus of the family. Following Oudemans (1936) and Fonseca (1948), *Macronyssus* replaces *Liponyssus* as the family type, and therefore the family name is changed accordingly.

It is shown that *Lepronyssus* Kolenati, 1858, and *Chiroptonyssus* Augustson, 1945, are synonyms of *Ichoronyssus* Kolenati, 1858.

PLATE IV

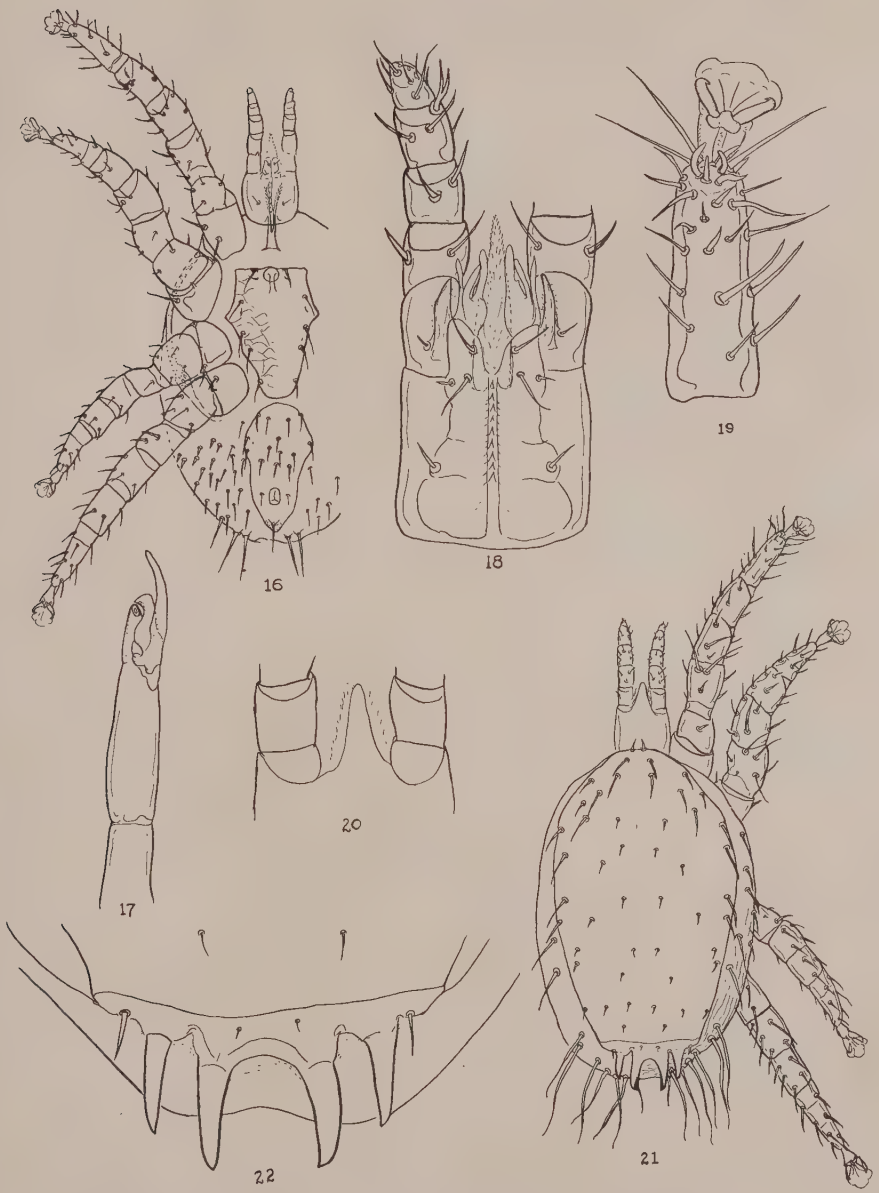

PLATE IV. *Ichoronyssus quadridentatus* new species, male.

FIG. 16, ventral view; FIG. 17, chelicerae; FIG. 18, ventral view of gnathosoma. *Ichoronyssus quadridentatus* new species, male. FIG. 19, tarsus I, dorsal view; FIG. 20, epistome; FIG. 21, dorsal view; FIG. 22, enlarged view of the posterior portion of the dorsal plate.

The genus *Spinolaelaps* Radford, 1940, is shown to belong in the family MACRONYSSIDAE and to be closely related to *Ichoronyssus*.

The species *Leiognathus sylviarum* Canestrini et Fanzago, 1877 is transferred to the genus *Bdellonyssus* Fonseca, 1941.

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A SURVEY OF COLORADO BAND-TAILED PIGEONS, MOURNING DOVES, AND WILD COMMON PIGEONS FOR *TRICHOMONAS GALLINAE*

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INTRODUCTION

The common pigeon (*Columba livia*), now a wild bird over much of the United States, is the ultimate source, in all probability, of the outbreaks of canker (trichomoniasis due to *T. gallinae*) which occur sporadically in chickens, turkeys, other pigeons, and various types of doves. The disease has been reported at various times from mourning doves (*Zenaidura macroura* subsp.), and a serious outbreak occurred in several southern states, particularly Alabama, during the spring, summer, and early fall of 1950, at which time these doves were said to have died by the thousands.

In an effort to learn more of the distribution of this parasite, 309 Colorado columbids were examined for its presence. These included 109 band-tailed pigeons (*Columba f. fasciata*), 100 western mourning doves (*Zenaidura macroura marginella*), and 100 common pigeons (*Columba livia*).

MATERIAL AND METHODS

The band-tails were wild birds trapped some 25 miles north of Colorado Springs. They were brought to the Springs, where they were examined, banded, and released. The mourning doves were collected within 100 miles of Colorado Springs, and the common pigeons were trapped in and about buildings within 15 miles of the Springs.

All these birds were examined by removing material from the pharyngeal portion of the mouth with curved forceps, particular attention being paid to the securing of material from behind the palatal flaps. This material was examined immediately in saline with the microscope. This procedure has, in the writer's experience, proved superior to the use of cotton swabs, mouth washings, and attempts to pipette material out of the mouth.

No cultures were made. It is possible that had such a procedure been employed the incidences of *T. gallinae* might have been slightly higher. The writer has found, however, that the careful examination of freshly removed oral material can hardly be improved upon. He has found birds to be positive by this procedure where cultures have failed.

Sincere thanks are extended to Clyde P. Matteson, Colorado Game and Fish Department, who managed the trapping of the band-tails.

OBSERVATIONS

The incidence of *Trichomonas gallinae* in all three of the columbid groups combined was 36.6 per cent.

Band-tailed Pigeons. These magnificent wild pigeons were trapped on a ranch

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west of Palmer Lake, Colorado, during the summers of 1948 and 1949. Of 109 individuals taken, 21, or 19.3 per cent, were positive for *T. gallinae*. Six birds examined in 1948 were retrapped in 1949. Two of them negative in 1948 were positive in 1949; one negative in 1948 was still negative in 1949; two which were positive in 1948 were still so in 1949; and one which was positive in 1948 had become negative by 1949. Although the trapping spanned a period from May until September, but one immature bird was taken. This was a youngster trapped 20/VI/48, the primaries of which were still in the blood.

No evidences of recovery from severe trichomoniasis, as indicated by loss of oral tissue, palatal fringes, etc., and no sick band-tails were noted in the 21 positive birds. No attempt was made to determine the relative pathogenicity of the strains of *T. gallinae* in these birds.

Western Mourning Doves. These birds, collected in 1948, '49, and '50, showed 23 positive of the 100 examined. Many were juveniles, and there appeared to be no correlation between age and incidence of *T. gallinae*. No special effort was made to test strain virulence from these doves, although some strains must have been fairly pathogenic, as indicated by the occasional loss of palatal tissue and the finding of one juvenile dying with extensive oral caseation.

Common Pigeons. Taken in traps and caught in buildings at night in and around Colorado Springs, also in '48, '49, and '50, these wild pigeons showed a 69 per cent incidence for *T. gallinae*. There was much more evidence of pathogenicity here than in the band-tails and doves, as loss of parts of the palatal fringes was common. A large majority of the birds taken from a particular bell tower in Colorado Springs showed these oral tissue losses, and their particular incidence of *T. gallinae* infection was 75 per cent. One young bird found dying in this colony was observed to have a huge caseous mass involving the base of the tongue and the floor of the mouth.

DISCUSSION

The infection rate of 36.6 per cent for wild Colorado columbids, the recent outbreaks of serious trichomoniasis in the south, and the presence of definitely pathogenic strains of *T. gallinae* in the Colorado birds, indicate the possibility of epidemics elsewhere, for in all probability the endemic rate for Colorado is not unique.

EFFECT OF *TRICHOMONAS GALLINAE* FROM DISEASED MOURNING DOVES ON CLEAN DOMESTIC PIGEONS

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INTRODUCTION

Trichomonas gallinae, pathogenic trichomonad of birds, was reported from captive mourning doves (*Zenaidura macroura*) and ring doves (*Streptopelia risoria*) by Cauthen (1936). It was described from a diseased wild mourning dove in Ohio by Harwood (1946), in North Carolina by McCulloch (1950), and in Colorado doves by Stabler (1950). The writer (1950) also reported it from the band-tailed pigeon (*Columba f. fasciata*) from Colorado.

The early spring, summer, and fall of 1950 saw an outbreak of dove trichomoniasis over most of the southern United States, with perhaps the greatest mortality occurring in Alabama. Haugen (1950) thought that the deaths might "run well into the thousands" in that state. What this might do to the mourning dove as a species is, of course, difficult to say at this moment. It seems certain that the parasite will be an important ecological factor indefinitely.

One wonders whether or not the trichomonads from these dead and dying doves could or could not establish themselves in clean (trichomonad-free) pigeons, and, if they could produce infections, whether or not they could harm the pigeon. Several investigators close to the sites of the outbreaks most generously supplied the writer with material from diseased doves, or the birds themselves. It is the result of placing the trichomonads from these sources into members of the writer's colony of *T. gallinae*-free common pigeons (*Columba livia*) with which the present paper deals.

MATERIAL AND METHODS

The trichomonads for experimentation were isolated from ill doves from a variety of sources.* Used as control were trichomonads from a healthy mourning dove collected at Colorado Springs, Colorado. As a control against the relative virulence of the dove flagellates, the writer infected a series of similar, *T. gallinae*-free pigeons with his very virulent Jones' Barn strain (Strain I) of *Trichomonas gallinae* (see Stabler, 1948a), originally isolated from a pigeon squab.

The recipients of the flagellates from these various sources were *T. gallinae*-free pigeons, some homers, some kings, some rollers. The organisms were dropped, in Ringer's solution, into the rear of the birds' mouths. There were no injections. Care was taken to insure that all the birds inoculated with the trichomonads from a particular source received approximately the same number of organisms each.

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*Dr. C. M. Herman sent a dove infected with trichomonads derived from a dove trapped at Laurel, Maryland. Dr. A. O. Haugen sent an infected dove which was trapped just east of Auburn, Alabama. He also sent two similar birds taken in Colbert Co., Alabama. Cultures from a dove were sent from the Alabama Polytechnic Institute, School of Veterinary Medicine, through the courtesy of Dr. W. S. Bailey. From the Wheeler Refuge, Decatur, Alabama, Biologist T. Z. Atkeson kindly forwarded a sick dove. The writer expresses his sincerest appreciation to these individuals, whose time and effort resulted in the arrival of viable trichomonads in Colorado Springs, in dove and culture.

When the dove was the source of the inoculum, the suspension for the five birds in a series was made up in one lot. This was then carefully divided so that each bird shared equally. By means of a Neubauer haemacytometer it was estimated that each bird's inoculum contained 50,000–100,000 *T. gallinae*. Comparable inocula were used throughout.

In those birds given the Jones' Barn strain, the inocula contained fewer organisms, ranging from 3,000–10,000 trichomonads each. These latter were from the body cavity, as this strain kills by causing extensive visceral caseation, especially in the liver. These visceral trichomonads are bacteria-free (Stabler and Engley, 1946), in striking contrast with those used directly from the various doves' mouths.

OBSERVATIONS

The trichomonads from the five sources of diseased doves, from the one healthy one, and the virulent (see the Discussion for an interpretation of this term) trichomonad strain from the pigeon were all placed in clean pigeons. Each of the dove

TABLE 1.—*Results of putting* Trichomonas gallinae *from mourning doves into clean domestic pigeons*

	Dove source	Pigeon no. 1	2	3	4	5
I.	Herman, Patuxent Refuge, Laurel, Md.	++	+++	++	+	+
II.	Haugen, Auburn, Ala.	++ ++	++	++ ++	++ ++	++ ++
III.	Atkeson, Wheeler Refuge, Decatur, Ala.	++	++	+	++	++ ++
IV.	Bailey, A. P. I., Auburn, Ala.	+++	++	++	++ ++	+++
V.	Haugen, Colbert Co., Ala.	+	+	++	++	+
VI.	Stabler, Colorado Springs, Colo.	0	0	0	0	0
	Jones' Barn control (pigeon strain)	++ ++	++ ++	++ ++	++ ++	++ ++

0 = no caseation; + = mild caseation; ++ = severe caseation; +++ = quite severe caseation, just short of death; ++++ = fatal infection.

strains was given to five birds; the pigeon strain has, at this writing (25/III/51), gone through twenty-seven successive pigeons, killing all but no. 19, which survived. The results are shown in Table 1.

It is realized that a series of five birds represents a rather small test. When numbers of experiments are being run simultaneously, however, it is no mean task to keep the supply of trichomonad-free squabs a step ahead of the demand. The number of birds per experiment was kept at a minimum, therefore, purely as an economy measure.

From the table it can be seen that the Auburn dove harbored a very virulent strain of *T. gallinae*. It killed four of its five pigeons and caused severe caseation in the fifth. The Decatur and A. P. I. strains killed only one bird each, though the latter was more pathogenic, very nearly killing two others. The strain from Maryland almost got one bird (no. 2), but was not too severe generally. The Colorado strain did not produce the slightest visible caseation, though all five birds became infected. The Jones' Barn virulent control strain has killed twenty-six of twenty-seven recipients in this series, death occurring at 9.3 days (average). See Stabler

(1948a) for a discussion of the Jones' Barn (Strain I) and Peregrine Falcon (Strain V) virulent strains used here as controls.

It is evident that the trichomonads from the mouth and crop of doves will infect clean pigeons. These infections may, further, result in the death of the pigeon. The dove strains vary in virulence in a manner comparable with that found by the writer (1948a) for the strains from domestic pigeons.

It has been shown that pigeons which survive a sublethal infection with *T. gallinae* derived from pigeons appear to be highly resistant to the otherwise quite virulent Jones' Barn strain (Stabler, 1948b). To see whether or not the survivors indicated in Table 1 were protected against virulent pigeon-derived *T. gallinae* by their previously acquired dove-derived *T. gallinae,* each bird in series I–V was given six drops of inoculum containing the virulent Jones' Barn strain. The results are presented in Table 2. Series VI was given the slightly less lethal Peregrine Falcon strain. Not a single bird indicated as having survived in Table 1 died when given these virulent strains (Table 2). Their dove infections had, in most cases, protected them completely.

TABLE 2.—*Results of putting virulent* Trichomonas gallinae *into survivors of the dove-*gallinae *infections from Table 1*

	Dove source	Pigeon no. 1	2	3	4	5
I.	Herman, Patuxent Refuge, Laurel, Md.	0	0	0	0	0
II.	Haugen, Auburn, Ala.	Dead	0	Dead	Dead	Dead
III.	Atkeson, Wheeler Refuge, Decatur, Ala.	0	0	0	0	Dead
IV.	Bailey, A. P. I., Auburn, Ala.	0	0	0	Dead	0
V.	Haugen, Colbert Co., Ala.	0	+	0	0	+
VI.	Stabler, Colorado Springs, Colo.	++	+	0	++	+

Note: Those birds indicated as "Dead" are the ones failing to survive, as shown in Table 1.
Birds from sources I–V received Jones' Barn strain as the virulent one. This strain has killed twenty-six of twenty-seven recipients as of 25 March 1951.
Birds from source VI received the Peregrine Falcon strain as the virulent one. This strain killed two of five birds, making the other three sick almost to the point of dying.

Only the pigeons with the Colbert Co., Ala., and the Colorado Springs dove strains showed any lesions whatever with the virulent pigeon strains, and these were severe in only two birds of the Colorado series. All recovered.

It was then wondered whether or not the pigeons protected by the dove-strain *T. gallinae* (Table 1), and harboring also the virulent, but now not killing, Jones' Barn *gallinae* (Table 2), 1) had actually killed off the virulent strain, 2) whether or not the virulent strain was present but now altered so that it was nearly avirulent and unable to cause more than mild pathology, or 3) whether the virulent strain was as potent as ever for clean birds, but unable to harm the protected ones. The following program was designed to shed some light on the above questions (Table 3).

The five pigeons surviving the Colbert Co. dove strain (Table 1) were chosen. They were given the Jones' Barn virulent strain, and subsequently showed mild caseation in two birds only (Table 2). These five birds now had a combination of the dove and the Jones' Barn strains of *T. gallinae,* the former generally mild, the latter quite virulent. This trichomonad combination was then given to five new birds, one bird infected from each of the five donor pigeons. This was repeated, making two infected birds from each donor, or a total of ten receiving the combina-

tion. From Table 3 it can be seen that the results were quite variable. The *T. gallinae* from donors no. 4 and no. 5 killed both their respective recipients. That from no. 1 produced only mild caseation in its two birds. The trichomonads from no. 2 and no. 3 barely affected one bird each, but no. 3's flagellates killed one bird, and no. 2's almost did.

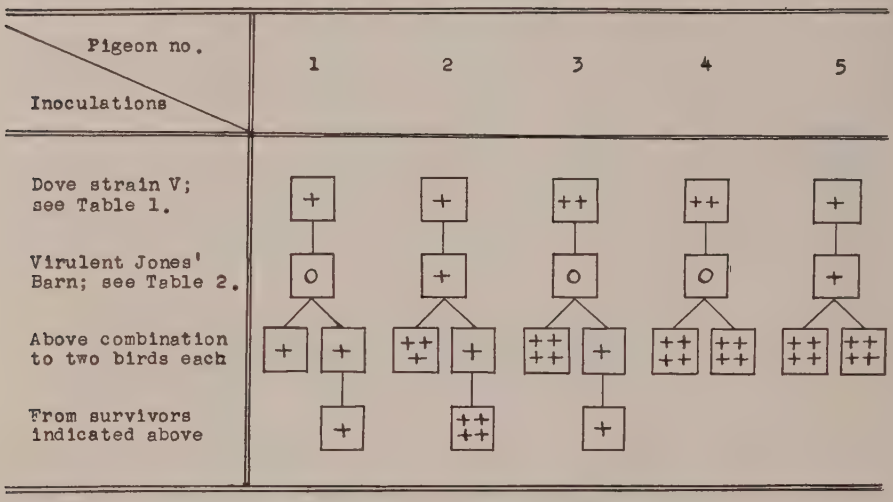

TABLE 3.—*Results of putting trichomonads from pigeons with the dove-Jones' Barn* gallinae *combination into clean domestic pigeons*

One may see that five of the pigeons were killed, one almost so, and four barely showed any caseation. None escaped altogether. The least affected member of the pairs receiving the combination from birds nos. 1, 2, and 3 were used as new donors for three new recipients. The results appear in the bottom row in Table 3. Again, they are quite variable. From nos. 1 and 3 the caseations were as mild as in the new donors immediately above them. From no. 2, however, another mild donor, the trichomonads killed the recipient.

DISCUSSION

The data recorded in Tables 1 and 2 support previously recorded observations to the effect that not only are there strains of *T. gallinae* which vary with respect to virulence for common pigeons (Stabler, 1948a), but that the survival of an attack by one of the less virulent strains of the organism is accompanied by an immunity which confers a high degree of protection against the future activities of virulent strains (Stabler, 1948b). It is interesting that strains from wild mourning doves behave in ways identical with those isolated from the common pigeon.

An explanation of the writer's use of the term "virulence" seems appropriate at this point. The Jones' Barn strain of *T. gallinae* kills nearly all pigeons which receive it, only an occasional bird surviving. As the inoculations with this strain were made from the body cavity, and therefore relatively bacteria-free, the subsequent lesions may reasonably be interpreted as being due solely to the trichomonads. Further, Stabler and Engley (1946) showed that bacteria isolated from caseations

themselves had no appreciable effect on the extent or the course of subsequent trichomoniasis. The Jones' Barn strain, then, became the yardstick of virulence, the effect of all other strains being measured against it. Virulence here, therefore, is a relative affair, with all reasonable control of variation being exercised.

It is true that no control was attempted over the organisms other than the trichomonads which accompanied the inocula derived from the mouths of the pigeons and doves. Such control is not, of course, exercised in nature, and there is considerable evidence that it would play a very minor role in the subsequent picture of the disease. It was felt, therefore, that the data reported herein represented a qualitative indication of circumstances which might occur in the wild.

The implications of the data presented in Tables 1, 2, and 3 are most significant as they apply to feral populations of mourning doves or other columbid birds. It is clear that a hen or cock bird which receives a highly virulent strain as its first infection of *T. gallinae* stands a good chance of dying of trichomoniasis, or of becoming so ill and weakened that it would fall easy prey to such natural enemies as the hawk, fox, rat, domestic cat, etc. It also might live long enough to pass its strain on to other members of its tribe.

A bird first becoming infected with a mild strain has the great advantage of protection with survival. Its future mate could not, then, cause it serious trouble by transferring to it a virulent strain.

What, perhaps, is of still greater implication is the fact that a bird harboring both an initially acquired mild strain and a subsequently acquired highly virulent strain may pass the latter on to a long series of clean mates or squabs with frequently serious, if not disastrous, results.

From Table 3 it is seen that thirteen birds received such a combination. Of these, none escaped caseation, six died outright, one barely survived, and six had mild lesions. If we imagine this as happening in the wild, which is not at all beyond the realm of possibility, these thirteen could conceivably have been the mates of one donor bird. There very likely would have been seven deaths. The six survivors, as indicated in Table 3, also could have passed their combined infections on to numerous luckless mates, with undoubtedly a repetition of the results. It is obvious that this could lead not only to a large series of deaths over a period of time, but could implement a wide spread of the virulent strains of *T. gallinae*. This admittedly is speculation. It does not seem too unreasonable, however, to suppose that such a mechanism might have operated in at least assisting in the final extermination of the Passenger pigeon (*Ectopistes migratorius*), and that it might result in a considerable reduction, locally at least, in the mourning dove.

SUMMARY

1. *Trichomonas gallinae* from five sources of diseased doves, and from one healthy one, were put into five trichomonad-free domestic pigeons each. The trichomonads from the healthy dove produced no lesions. Those from the sick doves produced some canker in all recipients. Some strains were quite pathogenic, causing severe caseation or death in most of the pigeons receiving them.

2. The pigeons surviving their infections of dove-derived *T. gallinae* were then given highly virulent pigeon-derived *T. gallinae*. Whereas the virulent strain killed twenty-six of twenty-seven successive trichomonad-free control pigeons, not

one of the survivors of the dove-infected pigeons became more than moderately ill when given this virulent strain.

3. The trichomonads from the five birds with the combined infections of the dove strain from Colbert Co., Ala. and the virulent pigeon-derived *T. gallinae* next were put into two clean pigeons each. Of the ten recipients, none escaped caseation, four were mildly caseous, one nearly died, and four were killed outright.

4. Three of the above birds which showed the mildest lesions were used as new trichomonad donors for one clean pigeon each. Two of these latter were again only mildly caseous, while the third one died.

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THE COURSE OF THE BLOOD-INDUCED *PLASMODIUM BERGHEI* INFECTION IN WHITE MICE

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The high incidence of sporozoite infection in *Anopheles dureni* Edw. led Vincke and Lips (1948) to the discovery of a new species of malarial parasite, *Plasmodium berghei,* in the tree rat, *Thamnomys surdaster.* The parasite has also been found infective to white mice (Vincke and Lips, 1948; Schneider, Decourt and Montézin, 1949; Vincke and Van den Bulcke, 1949; Thurston, 1950; Schneider and Schneider, 1950; and Schneider and Montézin, 1950), white rat, *Rattus rattus,* (Vincke and Lips, 1948; Vincke and Van den Bulcke, 1949a), cotton rats, (Rodhain, 1949), the field vole, *Microtus guntheri,* and the golden hamster, *Mesocricetus auratus,* (Adler *et al.,* 1950). Insect transmission of the infection has been obtained through the use of wild caught *Anopheles dureni* (Vincke, 1950); however, complete laboratory transmission by mosquitoes has not yet been successful.

Because of the possible importance of this parasite in chemotherapy of malaria it was decided to carry out a detailed study of the infection in several small rodents before embarking on a large-scale testing program. This investigation deals with the course of the blood-induced infection in the white mouse, *Mus musculus.*

MATERIALS AND METHODS

The KBG. 173 strain of *Plasmodium berghei* used in these investigations was furnished us through the kindness of Dr. A. Dubois, Director of the Prince Leopold Institute of Tropical Medicine, Antwerp, in July 1949 and has been maintained in this laboratory by blood passage.

In the present investigation three sets of 30 mice each were studied; each animal was approximately six weeks old. Each mouse received intravenously approximately 4,000 parasitized red blood cells. After inoculation the animals were examined daily for physical signs indicative of malarial infection. Beginning on the third day after inoculation, blood smears were made daily during the entire course of the infection. The blood smears were stained with Giemsa. Parasitemia was expressed as the percentage of parasitized red blood cells rather than according to the actual number of parasites present because of the common occurrence of multiple infections. A record was also kept as to the occurrence of segmenters and, whenever counting was facilitated by a distinct organization of the merozoites, of the number of merozoites produced per segmenter.

In order to ascertain the degree of anemia produced during the infection, red blood cell-counts were made just before inoculation and, generally, daily during the infection.

The length of an observation period expressed in days is relative to the number of complete days following the day in which the event took place. Thus, a patent

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period of six days refers to six complete days following the day on which the animals first became positive.

EXPERIMENTAL RESULTS

Of the 90 mice inoculated, 88 (97.8 per cent) became infected and all died of the infection. The first parasites appeared in the blood from three to six days after inoculation. In the entire series, 42 (47.7 per cent) animals first exhibited parasites on the third post-inoculation day, 19 (21.6 per cent) on the fourth, 14 (15.9 per cent) on the fifth, and 13 (14.8 per cent) on the sixth. The average mean prepatent period was 3.98 ± 0.12 (S.E. of mean) days. The parasitemia increased progressively during the first five days, the greatest percentage of animals reaching the peak in five to 13 days (average mean, 8.38 ± 0.40). The average daily parasite counts showed a steady increase from the onset of parasitemia until the fifth patent day, when 34 per cent of the red blood cells were infected; thereafter, a progressive increase did not occur and the same degree of parasitemia was more or less maintained until the end of patency (Figure 1). The patent period varied from four to 19 days (average mean, 10.34 ± 0.43). Death of most animals (53 or 60.2 per cent) occurred 24 hours after their peak parasitemia, 14 (15.9 per cent) died in two days, four (4.54 per cent) in three, 10 (11.4 per cent) in four, and seven (7.95 per cent) in five days.

Daily counts of the number of merozoites per segmenter showed that during the early course of the infection the range was three to 16 with an average of 8.04 ± 0.35. This number declined to an average of 5.52 ± 0.12, with a range of three to 12, on the third patent day, and then gradually increased to 12.8 ± 0.49, with a range of 12 to 14, on day 17 (Figure 1).

The effect on the erythrocyte count was marked. From an initial normal value of 9.82 ± 0.10 millions per cu. mm. a sharp decrease to 5.31 ± 0.31 occurred on the fourth day of patency. This was followed by a steady decrease until death occurred (Figure 1). The lowest average count was 1.17 ± 0.13 millions per cu.mm. on the fifteenth patent day. The sharp drop in the erythrocyte count coincided more or less with the first peak in the parasitemia and the lowest value in the merozoite mean per segmenter (Figure 1).

The physical examination of the infected animals showed that as the infection progressed the normal pink color of the eyes, ears, tail, and extremeties was replaced by a light yellowish color, the coat became ruffled, there was considerable loss of weight, and the animals became inactive. These signs were intensified at the terminal stage when labored breathing and somnolency occurred followed shortly by death.

DISCUSSION

Vincke and Van den Bulcke (1949) have shown that following subcutaneous inoculation of one or two drops of heavily parasitized blood, 75.4 per cent of the mice became infected after the first inoculation; the remainder became infected after a second or third inoculation. When the animals were given small intraperitoneal inoculations of parasitized blood, the prepatent period was two to three days with an infectivity rate of 98.4 per cent. The parasitemia in all cases increased progressively without intermittence until death. Thurston (1950) infected mice by inoculating parasitized blood either intraperitoneally or intravenously and showed that

irrespective of the route of inoculation the course of the infection was approximately the same. When five to 15 million parasites were injected intraperitoneally 20.7 per cent of the red cells were parasitized by the fifth day; death occurred in eight to 13 days. In regard to the phases of the infection studied by Vincke and Van den Bulcke and by Thurston our findings are in general agreement.

It is difficult to judge the accuracy of our results concerning the mean number of merozoites produced per segmenter or the average daily parasitemia during the latter course of the infection because after the fifth patent day the expected progression in the parasitemia, as determined by the parasite counts, did not occur. Also, the number of segmenters became progressively less and the organization of the merozoites less distinct as the terminal stage of the disease approached. Schneider

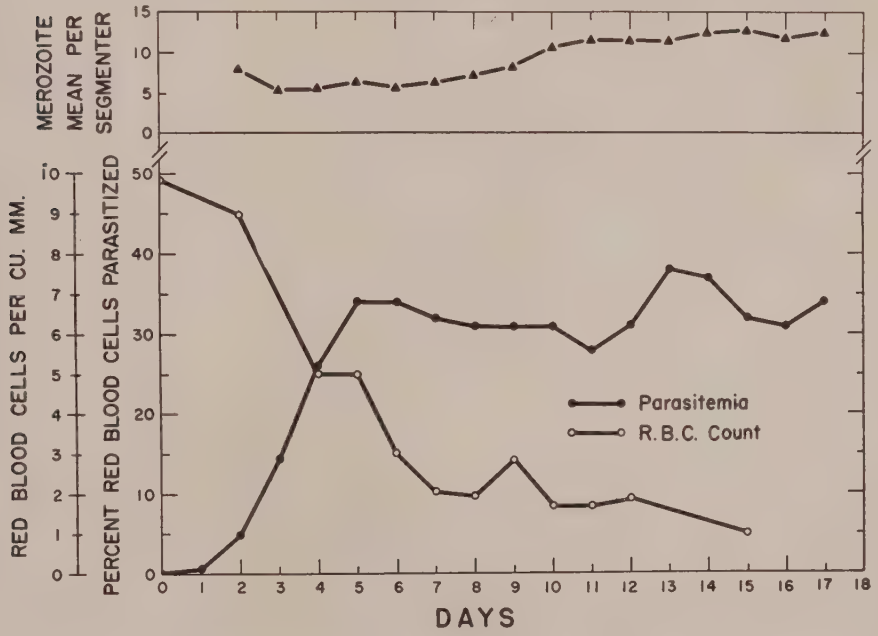

FIG. 1. The average per cent of parasitemia, average erythrocyte count per cu. mm. of blood, and the merozoite mean per segmenter in blood-induced *Plasmodium berghei* infections in white mice.

and Montézin (1950) reported a similar phenomenon in their studies; the parasitemia increased regularly and rapidly during the first five days but subsequently there were only small increases.

It seems, therefore, that the "plateau effect" in the parasitemia curve and the concomitant decrease in the number of intact segmenters is a natural phenomenon of this infection in the mouse and its occurrence may be due to a loss of these forms as a result of an increased fragility of the parasitized red cells. Shen *et al.* (1946) reported an increase in erythrocyte fragility in monkeys infected with *P. knowlesi* and put forward the idea that because of this phenomenon parasitized red cells were selectively destroyed *in vitro*. We have no data to show destruction of *P. berghei*-parasitized cells *in vitro* but smears of the parasitized blood gave convincing evidence

of their increased fragility because those made during the first days of patency rarely showed disrupted parasitized red cells but as the infection progressed more and more of these cells were seen. It is thought that these cells were broken during the process of smearing although some of this disruption may have occurred *in vivo* as suggested by Shen *et al.*

The lack of correlation between the degree of anemia observed in our investigations and the parasitemia produced after the sixth patent day might also be attributed therefore to this increase in the fragility of the infected red blood cells.

SUMMARY

1. The course of the blood-induced infection of *Plasmodium berghei* in white mice is characterized by a prepatent period of three to six days, with an average of 3.98 ± 0.12; a patent period of four to 19 days, with an average of 10.34 ± 0.43; a peak parasitemia at five to 13 days, with an average of 8.38 ± 0.40. Death, in most cases, occurred 24 hours after the peak; mortality rate was 100 per cent.

2. On the second day of patency the number of merozoites per segmenter ranged from three to 16 with an average of 8.04 ± 0.35; during the terminal stage of the infection the range was 12 to 14 merozoites per segmenter with an average of 12.8 ± 0.49.

3. As a result of the infection the normal red cell count of 9.82 million per cu. mm. dropped to 1.17 million per cu. mm. shortly before death.

4. Physical signs of the infection were ruffling of the fur, loss of the normal pink color of the eyes, ears, and extremities, loss in weight, and inactivity. These signs were intensified at the terminal stage of the disease when they were accompanied by labored breathing and somnolency.

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LEUCOCYTOZOON ANDREWSI N. SP., FROM CHICKENS OBSERVED IN A SURVEY OF BLOOD PARASITES IN DOMESTIC ANIMALS IN SOUTH CAROLINA[1]

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This survey was conducted in an experimental area in Clarendon County, South Carolina, where several types of data concerned with human malaria have been collected for a number of years. Despite the continued absence of any blood-positive cases of human malaria in the area for the 2 years since February 1949, wild-caught anophelines, upon dissection, are still found with oocysts and sporozoites. Both *Anopheles quadrimaculatus* and *Anopheles crucians* are involved. This survey was one of the investigations undertaken to locate the source of the malaria-like infections in the mosquitoes. Since practically all the anophelines for dissection are collected in stables and chicken houses, beneath dwellings, and about other farm buildings, domestic animals logically seemed to be possible hosts.

From Asia there are some reports in the literature of blood parasites belonging to the genus *Leucocytozoon* that were found in the domestic chicken. However, no records of such organisms occurring in this host outside the Eastern Hemisphere were located.

METHODS

The conduct of the survey in the field was routine after a day or two during which the most convenient handling procedure for each species of animal was developed. Generally, the owners were interviewed the day before it was desired to obtain the blood films in any certain section. Matters were expedited, particularly with mules, if the owners were present to aid with the animals.

Blood samples were secured from the domestic animals common in the area with somewhat different techniques. A small vein above the nostril of a mule or horse was punctured with a Hagedorn needle. When dealing with cattle, one of the small veins in the ear was pierced. A very small slit was cut in the margin of the ear in the case of the dogs and the same method was used for swine. On the ventral side of the wing of chickens and other poultry, a tiny vein was cut with the needle.

Both thick and thin blood films were made from the equine, bovine, canine, and porcine animals. From poultry, only thin smears were observed.

All films were allowed to dry overnight. The thin smears of mammalian blood were fixed in absolute methyl alcohol. The thin and thick films were stained for 45 minutes with a 1 : 50 dilution of Giemsa's stain buffered at pH 7.0. The thin films of avian blood, after drying, were fixed in absolute methyl alcohol and stained for 30 minutes in a 1 : 10 dilution of Giemsa's stain buffered at pH 6.8.

The slides were examined as soon as possible following preparation. A period of 20 minutes was spent carefully observing each slide under the microscope. The

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low power, the high dry, and the oil immersion objectives all were used in each examination. The use of the low power objective was extremely valuable in detecting organisms in rare numbers, such as *Leucocytozoon,* that otherwise might not have been noted.

For the demonstration of exflagellation of *Leucocytozoon,* fresh thin blood films were held in moist chambers in the refrigerator for a series of different intervals up to and including 2 hours. When desired, the slides were removed and stained as usual for examination.

OBSERVATIONS

Of the 300 equine animals examined, almost all were full grown mules (*Equus asinus* x *caballus*). There were very few horses (*Equus caballus*) and only 1 colt. No other young equines were noted in the area. The findings were negative except in 1 horse and 2 mules. In the blood of these 3, microfilariae were present. The microfilariae, although not positively identified, presumably were *Setaria equina.*

Four hundred bovines (*Bos taurus*) of all ages were sampled. In four, 1 young bull, 2 cows and 1 heifer-calf, *Trypanosoma theileri* was found in rare numbers. Microfilariae were observed in 8 others as follows: 6 cows, 1 young bull, and 1 heifer. Presumably the species of microfilariae was *Setaria cervi.*

A total of 106 pigs (*Sus scrofa*), young and old, was examined and no blood parasites were recorded.

There were 400 dogs (*Canis familiaris*) of all ages from which blood films were made in this survey. The only parasites noted in the microscopic examinations were the microfilariae of *Dirofilaria immitis.* Ninety-one animals or slightly less than 23 per cent were positive.

Poultry surveyed included 400 assorted adult domestic chickens (*Gallus gallus*), 13 adult domestic ducks (*Anas boschas*), 7 adult domestic geese (*Anser anser*), 2 domestic guinea fowls (*Numida meleagris*) and 10 domestic turkeys (*Meleagris gallopavo*), or 432 birds in all. No blood parasites were found in the ducks, geese, and guinea fowls. Every one of the turkeys had gametocytes of the protozoan parasite, *Leucocytozoon smithi.* In addition, 3 of the turkeys were infected with *Haemoproteus,* also a protozoan. Sixty-one chickens or 15.25 per cent have been observed as positive for a species of *Leucocytozoon.*

A description of the *Leucocytozoon* of the domestic chicken is given below. Only sexual forms were detected inasmuch as other stages of the parasite are not present, ordinarily, at least, in the peripheral circulation. Since other forms of *Leucocytozoon* have not been widely known, descriptions of various species have been largely limited to gametocytes and their host cells.

The macrogametocyte of the *Leucocytozoon* discovered in South Carolina (Fig. 1) is round, and its diameter averages 12 to 14 microns. The cytoplasm is dark blue, with dark purple granules frequently present. The nucleus is generally spherical and has an average diameter of 3 to 4 microns. Sometimes the nucleus is

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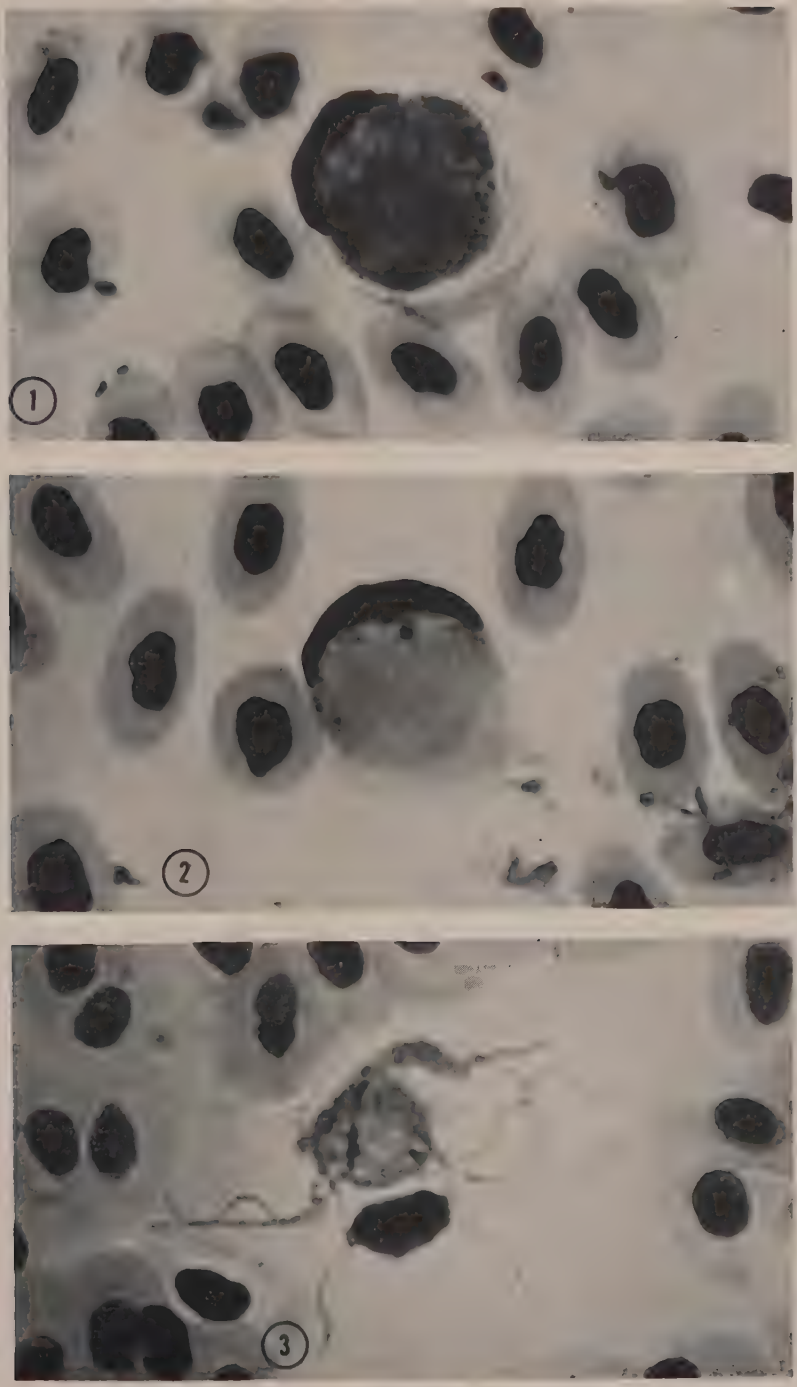
FIG. 1. Macrogametocyte of *L. andrewsi.* Magnification 2400×.
FIG. 2. Microgametocyte of *L. andrewsi.* Magnification 2400×.
FIG. 3. Exflagellation of the microgametocyte. Magnification 2400×.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elongated and measures approximately 5 × 2 microns. A distinct karyosome is present within the nucleus and is usually situated near the margin.

The microgametocyte (Fig. 2) also is round but somewhat smaller than the female gametocyte. The diameter of the male averages 10 to 12 microns. The male forms are far less numerous than the female. The cytoplasm of the microgametocyte is pale blue and the nucleus is pale pink. There is no definite shape characteristic of the nucleus, and it occupies most of the parasite. No karyosome is present in this nucleus, but occasionally red granules are scattered here and there.

The host cell is round, and those that enclose a female gametocyte average 15 to 17 microns in diameter. A host cell containing a male gametocyte is slightly smaller, averaging 13 to 16 microns in diameter. The dark red nucleus of the host cell is compressed to one side by the parasite. A narrow band of cytoplasm of the host cell is usually visible opposite the host cell nucleus and outside the margin of the parasite. The host cell nucleus is consistently present. The cytoplasm of the host cell appears pale pink.

Only round parasites and host cells have been noted in several infected chickens under observation for one year. No elongated types have been observed. While some fluctuations in the numbers of the *Leucocytozoon* gametocytes have been noted, these forms have been continuously present in the peripheral blood of infected fowl throughout the entire period of observation.

Exflagellation (Fig. 3) has been demonstrated in blood films retained for 2 hours in moist chambers in the refrigerator. At the time this took place, the gametocytes were of the same spherical shape, so they are definitely mature and not to be construed as merely young stages.

DISCUSSION

It was in the chicken alone that the observations of the survey resulted in a discovery possibly associated with the malaria-like infections in the anopheline mosquitoes. Chickens are very numerous in the area, and the genus *Leucocytozoon* seems to be related to malarial parasites. Studies are in progress to investigate any possible connection between the mosquito infections and this *Leucocytozoon*. Anophelines collected and dissected in the laboratory in recent years have been found to harbor sporozoites in the salivary glands at the rate of approximately 1 mosquito of every 1,300 brought in for examination. This rate apparently indicates that the unknown source of the anopheline infection is fairly extensive.

Morgan and Hawkins (1948) refer in some detail to studies carried on in the Western Hemisphere with different species of *Leucocytozoon* that occur in ducks and turkeys. These are characterized by spindle-shaped gametocytes, although some round forms do appear. It is typical of this genus that the gametocytes are fundamentally of 2 kinds, elongate and round. The host cells in which these sexual forms are found are shaped similarly. The elongated type is quite peculiar and distinctive. It is variously described as spindle-shaped or attenuated.

Published records noted in regard to species of *Leucocytozoon* in the domestic fowl originated in the Orient. Mathis and Leger (1909, 1910a, 1910b) observed the parasites in this host in Indo-China. These authors described both elongated and round gametocytes. In the case of the latter, it was emphasized that there was an inconsistency. This involved complete disappearance of the round forms for days or weeks followed by what seemed to be a relapse in that the parasites reap-

peared. After a few days, they vanished again from the peripheral circulation. This number of relapses was variable. Another prominent feature in regard to the round type was the usual absence of the nucleus of a host cell harboring a mature gametocyte. Mathis and Leger reported a few fowl with infections identified solely by round sexual forms, in the great majority only the elongated type was observed, and rarely there were infections in which both kinds were noted during the short time that the round gametocytes were present. In contrast, the spindle-shaped sexual cells persisted in the peripheral blood for long periods. Infections characterized by these attenuated forms were more frequently noted by the authors. The round gametocytes were considered to represent a species named *Leucocytozoon caulleryi,* and the elongated cells a different species designated as *Leucocytozoon sabrazesi.* Mathis and Leger were of the opinion that where both types of gametocytes were observed there was a mixed infection.

As noted by Morgan and Hawkins, in ducks infected with *Leucocytozoon simondi,* both round and elongated sexual cells may be found, and the same is the case with *Leucocytozoon smithi* of turkeys. Both of these situations are well known in North America.

More recently, Kuppusamy (1936) reported *Leucocytozoon* from chickens in Malaya which is near Indo-China. Both round and elongated sexual forms were observed together. According to the author, most of the mature gametocytes were spindle-shaped whereas the young forms were ordinarily round or perhaps oval.

From Sumatra, there is the record of Prowazek (1912) who gave an account of elongated sexual forms which he designated as *Leucocytozoon schüffneri* in domestic fowl.

Hamerton (1929) noted one case of *Leucocytozoon* infection in a chicken (*Gallus gallus*) in the London zoo when a blood film was taken from a dead fowl. The origin of the chicken was not mentioned in the report, and the parasite was not described.

With the discovery of *Leucocytozoon* in the domestic chicken in South Carolina, there exists a situation in which this genus of parasite is reported from this host only in widely scattered parts of the world. No information could be located dealing with any other occurrences in the Western Hemisphere.

The species of *Leucocytozoon* found in the domestic fowl in South Carolina is distinguished from the forms discussed above by its exclusively round gametocytes which are consistently evident in the peripheral circulation and by the regular presence of the nucleus of the host cell. Possible differentiations based on slight variations in size or staining tend to be controversial and do not warrant listing.

CONCLUSIONS

1. A total of 1,638 domestic animals of several species were examined for blood parasites in Clarendon County, South Carolina. No finding was made which could be correlated immediately with the unknown sporozoites occasionally observed in wild-caught anopheline mosquitoes in the same area.

2. The parasite described above from the domestic chicken (*Gallus gallus*) in South Carolina is designated *Leucocytozoon andrewsi* n. sp., in honor of Dr. Justin M. Andrews.

ACKNOWLEDGMENTS

The technical assistance of Miss Clarice Ashley is gratefully recognized.

The aid extended by Dr. M. D. Young and Dr. A. O. Foster in the search for relevant literature is appreciated.

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THE FAILURE OF THE RODENTICIDE WARFARIN TO INJURE ORIENTAL RAT FLEAS WHEN THE POISON IS FED TO THE HOST RAT

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From the standpoint of economy of operations, it would be desirable to have available a combination insecticide and rodenticide which might be applied in the same operation for the control of rodent-borne diseases. In general, especially in the face of an epizootic of plague, it has been necessary to control fleas first and then, in a separate coordinated campaign, to control the rats (Simmons and Hayes, 1948). It has been claimed by Wanson and Camphyn (1949) that partial control of rats may be achieved by the use of 50 per cent DDT but apparently this method has

TABLE 1.—*Results of tests regarding the effect of warfarin upon Oriental rat fleas when the poison in bait was fed to the host rat. The dosage of poison in bait was 0.10 mg. of warfarin per gram of corn meal.*

Test Number	Jar Number	Number and Source of Fleas in the Jar	Number of Rats Killed	Number of Days Fed	Number of Fleas	
					Removed from Rat and Jar When Feeding Stopped	Later Emerging from Culture
A	1	200 one-day-old fleas from the insectary.	1	5	100[a]	365
	2	200 one-day-old fleas from the insectary.	None (Control)	5	120	625
B	3	All adult fleas (approximately 100) taken from jar 1 and from the carcass of the rat in it.	5	25	300[a]	1,865
	4	All fleas (120) from jar 2 and the rat in it.	None (Control)	25	250	510
C	5	All adult fleas (approximately 300) taken from jar 3 and the last rat to die in it.	6	39	470	1,579
	6	All fleas (250) from jar 4 and the rat in it.	None (Control)	39	176	3,755
D	7	200 adult fleas that emerged from eggs in jar 3.	5	36	598	1,467
	8	200 adult fleas that emerged from eggs in jar 3.	5	32	620	1,524
	9	200 adult fleas that emerged from eggs laid in jar 4.	None (Control)	36	423	1,314

[a]—Approximate figure.

not been tested by other workers. Recently, a new anticoagulant rodenticide, warfarin, has been used successfully for the control of Norway rats at dosage levels as low as 0.05 mg. of the compound per gram of bait (Hayes and Gaines, 1950). The purpose of the present study was to investigate the possibility that warfarin might also control the Oriental rat flea, *Xenopsylla cheopis*, and thus permit a highly efficient and economical campaign against the host and vector of murine typhus and plague.

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[1] From Technical Development Services, Savannah, Ga.

Yellow corn meal poisoned at the rate of 0.10 mg. of warfarin per gram of meal was used as the only food of the experimental rats. The rats used as controls were fed poison-free yellow corn meal.

Adult white rats were confined to glass animals jars which were supplied with dry sand, ground rat feces, and shavings to provide an adequate culture medium for the insectary rearing of fleas. The rats were offered water overnight, each night. Only one rat was fed at a particular time in each jar but when a rat was killed by warfarin it was replaced by another, when required by the design of the experiment.

The sequence and results of these tests are shown in Table 1. It will be seen that the experimental fleas in jar 1 took their first blood meal from a warfarin-poisoned rat; the same fleas, when moved to jar 3, already had fed on a poisoned rat for 5 days before the second test began. Most of the fleas used in jar 5 were of a second generation whose parents had fed only on the blood of warfarin-poisoned rats. All of the fleas used in jars 7 and 8 were of a second generation whose parents had fed only on the blood of poisoned rats and they, of course, were also maintained on such rats. Controls in jars 2, 4, 6, and 9 were set up in parallel fashion but had no exposure to warfarin.

All of the warfarin-poisoned rats died within 4 to 10 days after feeding of the bait was begun. All rats that died were autopsied. Hemorrhages due to warfarin poisoning were present in all experimental animals and were sufficient to explain death in all except two. These two, the second rat to die in jar 8 and the third rat to die in jar 7, appeared, in addition, somewhat anemic indicating that excessive feeding by fleas may have hastened their death.

There was no indication that the health of *X. cheopis* adults or their reproductive capacity in the first or second generation was appreciably affected by maintaining the fleas constantly on rats poisoned by yellow corn meal bait containing warfarin at the rate of 0.10 mg./gm.

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EFFECTS OF ROENTGEN RADIATION ON *TRICHINELLA SPIRALIS*[1, 2]

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Radiation from various sources such as radium (Tyzzer and Honeiji, 1916), X-rays (Schwartz, 1921; Semrad, 1937; Evans, Levin and Sulkin, 1941; and Levin and Evans, 1942), ultraviolet rays (Storvens, 1942), short electric waves (Holl, Simon and Hector, 1940), and radioactive cobalt (Alicata and Burr, 1949) have been found to show a deleterious action on the reproductive cells of *Trichinella spiralis* and on their ability to infect the host animal. Schwartz (1921) sought to determine the practical application of X-rays in the destruction of trichinae in pork. When irradiated infected meat was fed to experimental rats, either the larvae were destroyed before reaching maturity or no young were produced by the adult female worms that did develop. Schwartz also pointed out that trichinae exhibited variations in the resistance to irradiation, since certain dosages proved to be destructive in some cases and not in others. He concluded that his observations were insufficient to warrant any definite conclusion concerning the feasibility of using X-rays to destroy trichinae in pork and that more observations were required to demonstrate whether this form of radiation could be depended upon to destroy trichinae.

In the reports which are available there appears to be considerable variation in the dosages reported necessary to produce certain effects on trichinae. According to Semrad (1937), trichina adults, exposed to 1200 r (roentgens) as larvae, failed to reproduce and were unable to effect subsequent muscle infection in rats. Levin and Evans (1942) reported larvae in the muscle of experimental animals following the feeding of larvae irradiated with 4000 r. In addition, Evans, Levin, and Sulkin (1941) indicated that in some experiments a dosage of 5000 r inhibited further development of the irradiated larvae. Alicata and Burr (1949), using cobalt 60, pointed out that following irradiation of trichinous meat with approximately 12,000 r, from 60 to 100 per cent of the adult females failed to produce fully developed embryos in experimentally infected animals.

The major purpose of this study was to secure, among other things, further data on the dependability of roentgen radiation in producing destructive or lethal action on trichinae, and to ascertain the dosage necessary to produce such action. It is believed that these data provide information for the possible application of radiation in the destruction of trichinae in pork.

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METHODS

The experiments reported in this study involved the irradiation of trichinous meat from rats and hogs and of trichina larvae (Plate I, Figs. 1, 2, 3) obtained from infected stock rats by pepsin digestion. In all cases, the animals from which the meat or larvae were obtained had been experimentally infected for at least 2 months. Each sample of meat used in irradiations weighed from 30 to 40 grams and was wrapped in thin cellophane. The samples were exposed directly to X-rays from a unit operating at 200 KVP and 20 ma current with a 0.25 mm. copper filter. The doses given ranged from 1000 to 30,000 r at a rate of approximately 150 r/minute. The dosage was measured in air with a calibrated Victoreen ionization chamber. The thickness of the meat sample irradiated never exceeded 20 mm. so that the variation in the dosage from the top to the bottom surfaces of the sample was about 10 per cent as determined by Andrews and Shore (1950). In experiments where dosages greater than 30,000 r were desired, soft X-rays from a Machlett type AEG-50T beryllium window tube (operated at 50 KV constant potential and 50 ma current with 0.56 mm. Dural filter) were used. The rate of delivery was approximately 2250 r/minute. The dosage of the soft X-rays was determined by the use of the chemical dosimetry method described by Andrews and Shore. Because of the limited penetrating power of these soft rays, the trichinous tissue used consisted of stretched-out pieces of heavily infected rat diaphragm or thin slices of pork. When isolated larvae were irradiated, a thin layer of larvae in a receptacle was used. The thickness of the layer or of the meat was not over 0.5 mm. so that the variation in the dosage from the top to the bottom of the sample was less than 3 per cent.

Soon after irradiation, the meat samples or the isolated larvae were fed to young rats weighing between 80 and 90 grams. These rats were usually starved for about 20 hours before infection and for 4 hours after infection. Control animals were always fed a similar quantity of non-radiated trichinous meat or isolated larvae from the same sample, and in all cases normal development and heavy infections took place. Following infection, the animals were sacrificed at various intervals and examined for presence of trichinae in the small intestines or muscles as noted in connection with each type of experiment. In examining the intestine, this organ was cut lengthwise and placed in a coarse 8-per-inch wire mesh strainer fitted within a sedimentation cone filled with warm (40° C.) saline solution. After about one hour the sedimented adult parasites were recovered and transferred to small droplets of saline solution arranged in rows on microscope slides. Each droplet contained one to three parasites and these were examined with a compound microscope. Samples of muscle from the animals were examined for larvae by press preparation methods. If no larvae were found, the entire carcass of each rat, after being skinned and eviscerated, was ground and digested in pepsin and further examined for larvae.

The Effects of Various Doses of Radiation on the Reproductive Power and Vitality of Trichinae

(a) *Observations when trichinous rat meat was used.*

In these experiments meat samples received dosages ranging from 1000 to 30,000 r. Two groups of 3 rats each were fed from each treated sample. In each group one rat was fed non-treated meat and served as control. Six days later the first group of rats, including the control, was sacrificed and the intestines examined

for adult trichinae. Whenever possible, 100 adult female trichinae were isolated on glass slides and examined microscopically for worm-shaped embryos. Females which did not show such embryos were recorded as sterile, even though many late cleavage stages were present.

Twenty-eight days following experimental infection, the second group of rats was sacrificed and the musculature of each animal was examined for trichina larvae by press preparation or by pepsin digestive methods.

The results of the above experiments are summarized in Table 1. This table shows that with increased radiation there is a corresponding increase in the percentage of sterility among the adult female worms (Plate I, Figs. 4, 5). Sterility in all the females was accomplished by a radiation dosage of 10,000 r, as evidenced in the absence of larvae in the musculature of rats infected with larvae exposed to this dosage. In addition to the data recorded in Table 1, 30 rats were fed trichinous meat irradiated with 10,000 r and 5 other rats were fed each about 20,000 isolated larvae irradiated with the same dosage. When these animals were sacrificed two months later no larvae were found in the musculature.

TABLE 1.—*Summary of infection of rats with irradiated trichinous rat meat. Results based on 3 separate trials in which a total of 18 rats (plus 6 controls) were used for each radiation dosage (9 rats for the intestinal infection and 9 for the muscle infection). X-ray machine operated at 200 kvp.*

Radiation dosage	Adults in the intestine 6 days after infection				Number of rats showing trichina larvae in the muscle 28 days after infection (total of 9 rats used)
	Percentage with fully developed embryos		Percentage sterile		
Roentgens	Range	Average	Range	Average	No.
1,000	100	100.0	0	0.0	9
2,000	86–100	97.8	0–14	2.2	9
3,000	84–100	94.8	0–16	5.2	9
4,000	69–100	86.9	0–31	13.1	9
5,000	40–100	65.9	0–60	34.1	9
6,000	2–25	12.9	75–98	87.1	9
7,000	0–17	7.3	83–100	92.7	9
8,000	0–8	1.7	92–100	98.3	2
9,000	0	0.0	100	100.0	1
10,000	0	0.0	100	100.0	0
15,000	0	0.0	(few adults)	100.0	0
20,000	0	0.0	(few adults)	100.0	0
30,000	No trichinae found				0

Table 1 also shows that only a few adult parasites were recovered from the intestine of rats which had been fed meat irradiated with 15,000 to 20,000 r. Actually, of 9 rats receiving meat exposed to 15,000 r, 7 showed from 1 to 7 adult parasites and 2 were negative. Of 9 rats receiving meat exposed to 20,000 r, 1 showed 3 adult parasites and 2 were negative. Six days after infection no trichinae were found in rats receiving meat exposed to 30,000 r.

With further reference to Table 1, one of the interesting features is that the degree of sterility among adult female worms is found to increase logarithmically with the dosage (Fig. 1). Phenomena of this kind are well known in connection with the irradiation of various kinds of cells and organisms (Lea, 1947). The curve in Fig. 1 shows that theoretically a dosage of about 6200 r should have given approximately 100 per cent sterility among the female worms. On the other hand there is a break at 6000 r and the end point is reached at 9000 r. Although the significance of these results is not clearly understood, the findings strongly suggest that some of the egg cells in trichina worms appear to be more resistant to radiation than the average

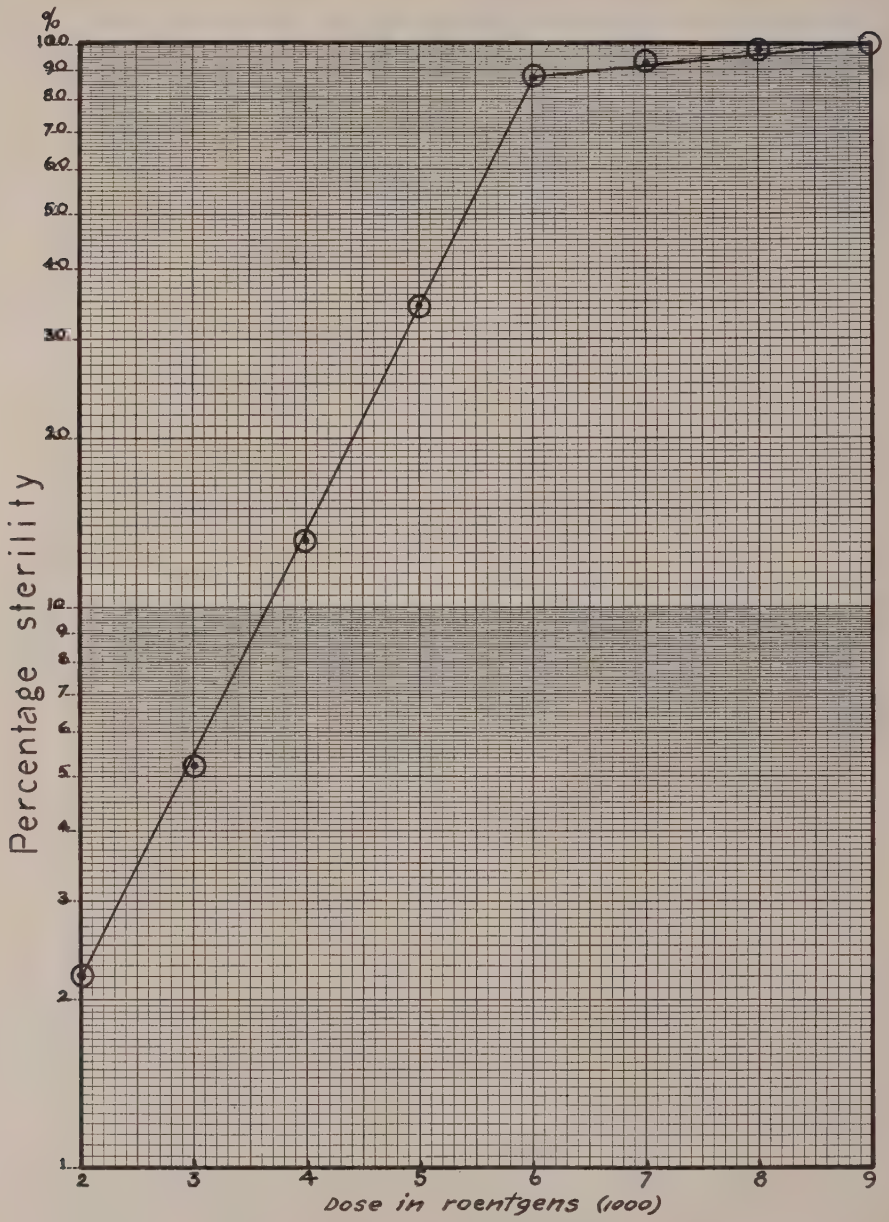


FIG. 1. Yield in percent of sterility among adult female trichinae exposed as larvae to various radiation doses.

egg cells. Such is illustrated in Plate I, Fig. 10. Whether or not such a difference in resistance of these cells is biological or due to the difference in the number of X-ray quanta absorbed remains to be determined.

Subsequent to the above findings, it was decided to determine the effects on trichina larvae exposed to 30,000 r. In this experiment, 12 rats were infected and 2 were sacrificed daily for a period of 6 days. Of the first 3 rats examined 24 hours after infection, all showed trichina larvae in the small intestine. These larvae had undergone some development as manifested by molting and presence of rudimentary copulatory appendages at the posterior end of the males. The remaining rats, which were killed from 2 to 6 days after infection, showed no trichinae in the intestine. Following the above results, 12 additional rats were each fed approximately 20,000 trichina larvae which had received a radiation dose of 30,000 r. In 2 of these rats examined 24 hours after infection, 94 and 120 trichina larvae, respectively, were recovered from the intestinal tract. In 2 other rats sacrificed 48 hours after infection, 2 and 5 larvae, respectively, were recovered. The remaining rats, which were sacrificed from 3 to 6 days following infection, showed no trichinae.

TABLE 2.—*Results of infection of rats with trichinous meat or with isolated larvae which had been exposed to high irradiation dosages. The rats were sacrificed 24 and 48 hours after infection. (X-ray machine operated at 50 kvp.)*

Radiation dosage	Location of larvae	Number of larvae found in the small intestine at autopsy	
		At 24 hours	At 48 hours
Roentgens			
100,000	Encysted in diaphragm	30	0
100,000	" " "	45	0
100,000	" " "	32	0
200,000	" " "	10	0
200,000	" " "	2	0
200,000	" " "	0	0
300,000	" " "	1	0
300,000	" " "	0	0
300,000	" " "	0	0
400,000	" " "	0	0
400,000	" " "	1	0
400,000	" " "	0	0
500,000	" " "	0	0
500,000	" " "	0	0
500,000	" " "	0	0
100,000	Isolated (20,000)	3,500	0
100,000	" "	1,200	0
200,000	" "	2,000	0
200,000	" "	740	0
300,000	" "	175	0
300,000	" "	140	0
400,000	" "	32	0
400,000	" "	48	0
500,000	" "	4	0
500,000	" "	0	0
600,000	" "	10	0
600,000	" "	0	0
700,000	" "	0	0
700,000	" "	0	0

Further observations on the effects of radiations above 30,000 r on trichina larvae encysted in rat diaphragm and on those which had been isolated was made with the use of soft X-rays as already mentioned. It was roughly estimated that each piece of diaphragm used contained approximately 1000 larvae. The results of observations in which the larvae were given doses varying from 100,000 to 700,000 r are summarized in Table 2. These data show that some of the larvae which had been exposed to as high as 600,000 r were alive in the intestinal tract 24 hours after infection. These larvae, however, were soon eliminated from the intestines as evidenced by their absence in rats examined 48 hours after infection. None of the larvae exposed to doses of 700,000 r was found in the intestinal tract of rats sacrificed 24 hours after infection. The ability of some of the larvae to become established in the intestine of the host for 24 hours and their inability to

remain much beyond that period may possibly be explained by delayed effects of radiation. It is well known that the visible effects of irradiation are usually not immediate but that changes follow a latent period which varies with dosage and other factors. In this connection, Packard (1931), explained that following irradiation, an organism may continue in an apparently normal condition and the effects are not noted until a major morphological or physiological change occurs such as gastrulation, hatching or metamorphosis. Among trichinae, profound morphological and physiological changes do occur during the first day of larval development in the intestine of the host, and it is possible that the deleterious effects of radiation follow some of these changes.

The above results indicate that the amount of radiation necessary to inhibit the formation of worm-shaped embryos in the uterus of the adult female worms is apparently higher than that reported by Semrad (1937), and Evans, Levin, and Sulkin (1941) and referred to in the introductory statements. These investigators, however, irradiated isolated trichina larvae in celluloid dishes rather than in trichinous tissue. With the possibility that the method used was responsible for

TABLE 3.—*Summary of infection of rats with irradiated trichinous pork. Results based on 2 separate trials in which a total of 12 rats (plus 4 controls) were used for each radiation dose (6 rats for the intestinal infection and 6 for the muscle infection). X-ray machine operated at 200 kvp.*

Radiation dosage	Adults in the intestine 6 days after infection				Number of rats showing trichina larvae in the muscle 28 days after infection (total of 6 rats used)
	Percentage with fully developed embryos		Percentage sterile		
Roentgens	Range	Average	Range	Average	No.
5,000	46–65	56.2	35–54	43.8	6
6,000	9–26	14.3	82–92	85.7	6
7,000	0–6	2.8	94–100	97.2	6
8,000	0–2	0.3	98–100	99.7	3
9,000	0	0.0	100	100.0	1
10,000	0	0.0	100	100.0	0
15,000	0	0.0	100	100.0	0
20,000	No trichinae found				
30,000	No trichinae found				

the difference, the writer exposed celluloid dishes containing isolated trichina larvae to various dosages of X-rays ranging from 1000 to 10,000 r (X-ray unit operated at 200 KVP). Subsequently, 2 rats were fed with larvae from each dosage. When the rats were sacrificed 6 days later, the average percentage of sterility among the female worms was as follows: 1000 r, 0.0 per cent; 2000 r, 2.5 per cent; 3000 r, 4.5 per cent; 4000 r, 19.0 per cent; 5000 r, 27.0 per cent; 6000 r, 72.5 per cent; 7000 r, 89.0 per cent; 8000 r, 95.0 per cent; 9000 and 10,000 r, 100.0 per cent. These percentages of sterility fall, for the most part, within the range of sterility recorded in Table 1, and indicate therefore that approximately similar results were obtained irrespective of the method used in irradiating the larvae.

(b) *Observations when trichinous pork was used.*

Some of the above experiments were repeated with the use of trichinous pork radiated with dosages varying from 5000 to 30,000 r. The results, which are summarized in Table 3, were in general similar to those found when the trichinized rat meat was used (Table 1). Furthermore, no live larvae were found in the intestines of 3 rats 24 hours after they had been fed thin slices of trichinous pork irradiated with 700,000 r.

Effects of Radiation on Encysted Trichinae in Pork at Freezing Temperature

Since temperature may be a factor in the action of radiation on trichinae, an experiment was conducted to determine whether, in animal feeding experiments, the same results would be obtained from trichinous pork irradiated at freezing temperature and at room temperature. The dosage of 9000 r was chosen because in previous experiments (Table 1) it was found to be the lowest dose that would produce approximately 100 per cent sterility among female worms. In this experiment, pieces of trichinous pork were packed in a small square cardboard box 1.5 inches wide and 1 inch high and placed in a 0° C. refrigerator overnight. The box was then immediately transferred to the center of another square plywood box 6 inches wide and 2.25 inches high with a removable wooden cover. The wooden box was then fully packed with crushed ice in which a few pieces of dry ice were added to prevent the regular ice from melting quickly. After the wooden box was covered it was exposed to 200 KVP X-rays and a dosage of 9000 r was administered. Just before and after irradiation, the temperature of the meat was ascertained by inserting a thermometer into the meat through small apertures made on the sides of the wooden and cardboard boxes. The temperature of the meat at the beginning and end of irradiation was found to be 0° C. The dosage of radiation which was to be administered under the above conditions was determined with the aid of a Victoreen ionization chamber which, protected by a rubber membrane, was inserted in the ice-packed box in the same manner as the thermometer.

Following irradiation, samples of the meat were fed to 3 young rats. In addition, 3 other rats were fed trichinous pork which had received a radiation dosage of 9000 r at room temperature (24° C.). Six days following infection, all 6 rats were examined and the adult trichinae were recovered from the intestinal tract. Microscopic examination of 100 female worms from each of the rats revealed all to be sterile, indicating that the radiation administered at the low temperature was as effective as when that administered at room temperature.

The Effects of Radiation on the Degree of Infection in the Host

The evident reduction in the number of adult parasites recovered from the intestinal tract of rats following irradiations of 15,000 to 20,000 r (Table 1) led to a series of experiments to determine the number of parasites which would become established in the host following infection with larvae exposed to 3000, 5000, 7000, and 9000 r. Following each exposure, about 2000 larvae from each treatment were fed to each of 6 rats. Similar numbers of non-irradiated larvae were fed to 6 rats which served as controls. Three rats receiving larvae from each treatment, including the control, were killed after 6 days to determine the number of parasites present in the small intestine. Three remaining rats were killed 2 months after infection to determine the number of larvae in the musculature. The results of these experiments, presented in Table 4, show that with increased irradiation there is a decrease in the number of parasites found in the intestine and muscle of the experimental animals. These results are in accord with the report of Levin and Evans (1942), who noted similar reduction of parasites in rats receiving larvae exposed to various increasing radiation doses.

Effects of Radiation on the Reproductive and Other Tissues of Trichinae

The observations made in this study confirmed those of Schwartz (1921) in that radiation was shown to exert a selective and destructive action on the gonads of trichinae. Although no apparent morphological changes are noted in larvae immediately after radiation, deleterious action, especially of the reproductive organs, is later noted in the parasites which mature when fed to susceptible hosts. The degree of damage produced at a given dosage is, however, not uniform. This may indicate that these parasites vary in their resistance to radiation, or that there is a variation in the amount of radiation which they absorb. The ovary of the irradiated adult females often shows shrinkage and malformation (Plate I, Fig. 13). In many cases the females show considerable reduction in the number of fully developed embryos (Plate I, Fig. 11) and in the number of egg cells present in the uterus (Plate I, Figs. 6, 7, 8, 9). The presence of segmenting egg cells is usually rare in adult female parasites (Plate I, Fig. 10) which, as larvae, were exposed to a radiation dose of 8000 r or more. In irradiation with 10,000 r the egg cells show many granules and are usually unable to segment.

Table 4.—*Degree of trichina infection in rats following the feeding of approximately 2,000 larvae exposed to various radiation dosages. Rats in group 1 were sacrificed 6 days after infection and rats in group 2 sacrificed after 2 months*

Group 1 (intestine)		Radiation dosage	Group 2 (muscle)	
Number of adults recovered	Ratio[1]		Number of larvae recovered	Ratio[1]
		Roentgens		
1,123	0.6	0 (control)	410,000	205
1,025	0.5	0 "	288,000	144
1,228	0.6	0 "	444,000	222
718	0.4	3,000	300,000	150
695	0.3	3,000	232,000	116
953	0.5	3,000	342,000	171
751	0.4	5,000	208,000	104
551	0.3	5,000	160,000	80
762	0.4	5,000	240,000	120
644	0.3	7,000	16,000	8
652	0.3	7,000	23,000	11
517	0.3	7,000	14,000	7
118	0.1	9,000	0	0
131	0.1	9,000	0	0
92	0.1	9,000	0	0

[1] Ratio equals number of worms recovered divided by number of larvae fed.

Normal copulation apparently takes place among irradiated parasites, at least among those receiving doses up to 7000 r. This is evidenced by the presence of some sperm cells in the seminal receptacles of adult females. Most female worms which have been exposed to a dose of 8000 r or more show very few or no sperm cells in the seminal receptacles.

The reproductive organs of the adult male trichinae appear to show little evidence of degeneration following irradiation. There is, however, considerable reduction in the number of sperm cells present in the seminal vesicle.

With increased exposure to roentgen rays the cuticle of the parasites, especially that of the females, shows wrinkling and abnormal thickenings along various points of the posterior half of the body. This leads to an irregular appearance of the body wall (Plate I, Fig. 12).

Roentgen radiation, in addition to producing the above mentioned tissue damage, results in stunting of the parasites (Plate I, Fig. 14). The average length of

50 adult female worms, exposed as larvae to a dose of 10,000 r, was found to be 1.2 mm., whereas that of 50 normal adult females was 2.7 mm.

Observations on Possible Recovery of Trichinae from the Effects of Radiation

Cook (1939) demonstrated that *Ascaris* eggs would not embryonate if allowed to develop at 25° C. soon after irradiation. But if these eggs were kept inactive under refrigeration for several weeks before allowing them to develop, they would embryonate. It was therefore decided to determine whether irradiated trichina larvae would recover if maintained under refrigeration for several weeks before being fed to susceptible animals. In these studies, carried out in two separate experiments, trichinous pork was exposed to a radiation dosage of 9000 r and was then kept under refrigeration at 0° C. for 4 weeks. Following this period the meat was fed to 6 rats and the infection allowed to progress for 6 days. Examination of 100 adult female trichinae recovered from these rats revealed sterility and atrophy of the ovarian structure similar to that seen when the larvae were fed to rats soon after irradiation. These observations therefore indicate that the 4-week rest period following irradiation did not reduce the lethal effects of irradiation on the reproductive cells of trichina worms.

Summary and Conclusions

1. Infective trichina larvae exposed to roentgen radiation at a dosage of 10,000 r failed to produce young when fed to susceptible hosts. This inability of the parasite to produce young insured the host against subsequent muscle infection.

2. Following irradiations of 15,000 to 20,000 r, a few larvae were able to reach maturity in the intestine of the host. No larvae reached maturity following irradiations of 30,000 r. The latter larvae, however, underwent partial development in the host during the first 48 hours of experimental infection, after which all were eliminated from the intestinal tract.

3. At irradiations from 100,000 to 600,000 r some live larvae were found in the intestinal tract of the host up to 24 hours after infection. However, no larvae were found in the intestinal tract 48 hours after infection.

4. Irradiation of about 700,000 r completely destroyed the power of all trichina larvae to become established and develop in the intestine of the host. Such larvae were quickly eliminated with the feces of the host during the first 24 hours of experimental infection.

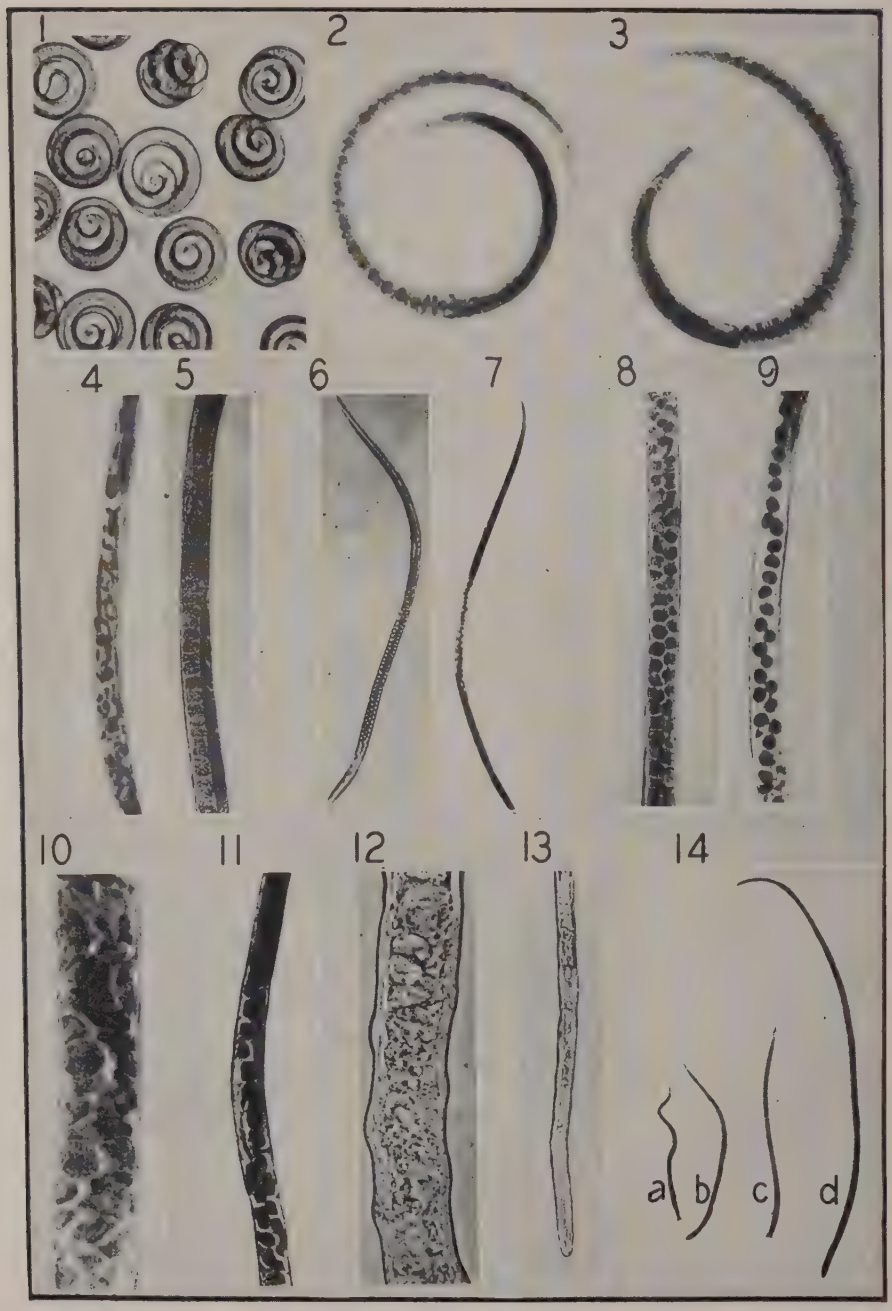
5. With increased irradiation, a gradual reduction in the number of adult parasites found in the intestinal tract and in the musculature of the host was evident.

6. The effects on trichinae were similar whether irradiated at 0° C. or at room temperature (24° C.).

7. Trichinae were found to show no recovery from the effects of radiation when maintained under refrigeration for one month following irradiation and subsequently fed to susceptible hosts.

8. Some of the evident morphological changes in irradiated trichinae were: shrinkage and degeneration of the ovary, inability of egg cells to undergo complete cleavage or to produce worm-shaped embryos, production of cuticular thickenings in the body wall, and stunted growth.

PLATE I



9. The laboratory tests indicate that roentgen radiation in sufficient quantity is an effective and dependable method for the destruction of trichinae in meat.

10. The practical application of roentgen radiation under commercial condition at the present time is questionable since an extremely high amount of radiation is necessary to destroy trichina larvae, and furthermore, it is not readily possible to irradiate large amounts of meat.

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Plate I. *Trichinella spiralis*.

Fig. 1. Normal infective larvae isolated from infected muscle. State at which larvae were exposed to roentgen radiation.

Fig. 2. Female infective larva showing degree of gonad development at the time of irradiation.

Fig. 3. Male infective larva showing degree of gonad development at the time of irradiation.

Fig. 4. Normal adult female, recovered from the intestine of a rat 6 days after experimental infection, showing fully developed worm-shaped embryos in the uterus.

Fig. 5. Adult female, recovered from the intestine of a rat 6 days after experimental infection. Parasite showing undeveloped embryos in the uterus resulting from exposure, as larva, to a radiation dose of 4,000 r.

Fig. 6. Normal adult female, recovered 6 days after infection. Note abundance and distribution of egg cells in the uterus.

Fig. 7. Adult female, recovered 6 days after infection, showing decreased number of egg cells in the uterus. This resulting from exposure, as larva, to 4,000 r.

Fig. 8. Same as 6 (central area magnified).

Fig. 9. Same as 7 (central area magnified).

Fig. 10. Central area of adult female showing 2 egg cells in late cleavage stage surrounded by other unsegmented egg cells. This resulting from exposure, as larva, to 8,000 r.

Fig. 11. Central area of adult female, recovered 6 days after infection, showing a single fully developed embryo. This resulting from exposure, as larva, to 6,000 r.

Fig. 12. Abnormal cuticular thickenings along the posterior portion of an adult female resulting from exposure, as larva, to 10,000 r.

Fig. 13. Posterior half of adult female showing degeneration of the ovary, resulting from exposure, as larva, to 7,000 r.

Fig. 14. Size of normal adult male (c) and female (d), as compared with adult male (a) and female (b) which, as larvae, had been exposed to 10,000 r.

A NEW SPECIES OF *ANOPHELES* FROM THE PHILIPPINE ISLANDS (DIPTERA: CULICIDAE)

LLOYD E. ROZEBOOM*

In the Oriental Region, the *umbrosus* group of *Anopheles* species belonging to the *Myzorhynchus* series is characterized by the absence of true palmate hairs in the larva. In the Philippine Islands, a member of this group was described by Russel and Baisas (1934, 1936) as a possible variety of *A. baezai* Gater, 1933; Baisas (1936) later elevated this form to specific rank and named it *A. gateri.* However, recently Reid (1950) has shown that *A. gateri* is a synonym of *A. baezai.*

In order to study variability among anopheline species, the writer has often reared progeny from isolated females taken in their resting places or by trapping. During 1945, several females captured in a trap baited with a calf, at Osmena, Samar, Philippine Islands, appeared to be *A. baezai* (identified at the time as *A. gateri*), but they produced larvae which did not conform to that species, thus suggesting a new form. As the differences between *A. baezai* and the new form appear to be consistent, the purpose of this paper is to present a description of the latter. Because of the insular nature of the distribution of this mosquito, which has several close relatives in the Malay Peninsula and neighboring regions, a name based upon the type locality would appear to be appropriate.

Anopheles (*Anopheles*) *samarensis,* new species

Female.—A fairly large, dark colored anopheline. *Head:* Frontal tuft white, vertex with white scales centrally, dark laterally. Palpi shaggy, entirely dark. Proboscis shaggy, dark; labella paler; proboscis and palpi about as long as the fore femur. Pharynx (Fig. 2) without a well defined pigmented area on posterior hard palate and without the pigmented patch as seen in *A. umbrosus;* anterior margin of posterior hard palate concave and irregular. Pharyngeal armature absent except for two small pointed denticles on each side at base of lateral flange. *Thorax:* Prothoracic lobe with some dark scales at upper margin and scattered setae; posterior pronotum dark, bare. Scutal integument dark brown, central portion grey-pollinose; anterior promontory with some very narrow pale scales centrally and with lateral tufts of dark scales; vestiture of remainder of scutum consisting of short, narrow, curved, golden setae. Scutellum pale brown, marginal setae short, pale. Propleuron with 2 setae. Mesopleura dark brown, without scales, except sometimes for a few at center or upper margin of the mesepimera. Upper mesepimeral setae (subalar) numbering from 5 to 12, arranged in a single row, an irregular double row, or a patch, with one or two associated scales, or these scales absent. Wing (Fig. 1) with costa and vein 1 mainly dark, subcostal white spot present but small, usually involving only the costa but sometimes including a few white scales at apex of subcosta; apical white spot small, involving costa and apices of veins 1 and 2.1; sectoral white spot small, present only on vein 1, and sometimes reduced to a few white scales. Vein 2 with stem dark, 2.1 dark except at apex; 2.2 dark, with a definite white spot or at least a paler area at middle. Vein 3 white with a basal dark spot. Vein 4 with stem dark basally, mixed white and dark scales centrally, the remainder white except for a dark spot at the fork and at apex of 4.2. Vein 5 mostly white, with a dark spot at base which does not extend half way to the fork, but often with scattered dark scales all the way to the fork; 5.1 with a small dark spot about one-fourth the distance from the fork and another spot at apex which may involve almost the apical half of 5.1; 5.2 with a dark spot at apex. Vein 6 white, with a dark spot at the middle and another at the apex; sometimes with a few dark scales at the base. A pale fringe spot present at apex of the wing, extending between the tips of veins 2.2 and 3. Halter dark. Femora of fore legs swollen at base, dark, somewhat paler

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below at base; mid and hind femora dark outwardly, paler on inner surfaces; tibiae dark, but hind tibiae with an apical white spot; tarsi of all legs dark, a very narrow apical white ring or spot present on segments I, II, usually III, and sometimes IV, somewhat more distinct on hind legs than on fore and mid legs. *Abdomen:* All segments dark and entirely without scales; cerci without scales.

Male.—Coloration similar to that of the female. Palpi dark. *Terminalia:* Sidepiece cylindrical, clothed with setae and dark scales outwardly, with two parabasal spines, the dorsal one almost twice as long as the ventral spine. Clasper a little longer than the sidepiece, with a short, stout, terminal spine. Dorsal (outer) claspette lobe (Fig. 3) with three filaments fused to form a club; ventral (inner) claspette lobe clothed with short setae, and with one long and one shorter setae at apex. Mesosome (Fig. 4) strongly curved dorsad, with 4 or 5 pairs of unserrated leaflets at apex, the upper leaflet the largest and succeeding ones progressively smaller. Lobes of ninth tergite about four times longer than wide, digitiform (Fig. 5), slightly clubbed at apex (Fig. 6), or short and strongly clubbed (Fig. 7).

Larva.—Head: Antenna cylindrical, inner and ventral surfaces with short spines, which are more numerous in central portion of the shaft; antennal hair 11 situated before middle, with 6–12 lateral branches, extending beyond apex of shaft; terminal hair 10 fine, 2–5 branched; one of the sabres pointed, the other with the tip rounded and frayed. Inner anterior clypeal hairs (Fig. 8) long, single, sometimes with a few lateral spicules, especially near tip, inserted close together so that their tubercles are usually contiguous; outer clypeal hairs about as long as inner clypeals, usually single, sometimes with one or two lateral spicules, occasionally split into 2–5 branches, (Fig. 9). Post clypeal hairs (Fig. 8) minute, single or double. Frontal and subantennal hairs long, feathered. Inner occipitals small, 2–5 branched, outer occipitals small, 1–4 branched. *Thorax:* Anterior submedian hairs (Fig. 10) with small, separated tubercles, hair 1 small, split into 2–4 branches, hair 2 with a slender shaft and about 8 lateral branches, hair 3 single, small. Metathoracic hair 1 with 4 or 5 small, hair-like branches. Prothoracic pleural group with three long and one small single hairs; mesothoracic pleural group with two long single hairs, one single hair about one-third the length of the long hairs, and one minute hair, methathoracic pleural group with two long single hairs, one short, 2–3 branched hair, and one minute hair. *Abdomen:* Anterior tergal plates roughly elliptical, about one-third as wide as the segments, that on segment VIII larger than the others. Palmate hairs completely absent; hair 1 (Fig. 11) with a slender shaft and about a dozen long lateral branches. Lateral hair 6 with a main stem and 6–10 lateral branches on segment IV (Fig. 12), split at base into 2 or 3 branches on segment V (Fig. 13), and with 6–10 branches on segment VI. Pecten with a fairly regular alternation of one long and one short unserrated spines. Saddle hair single.

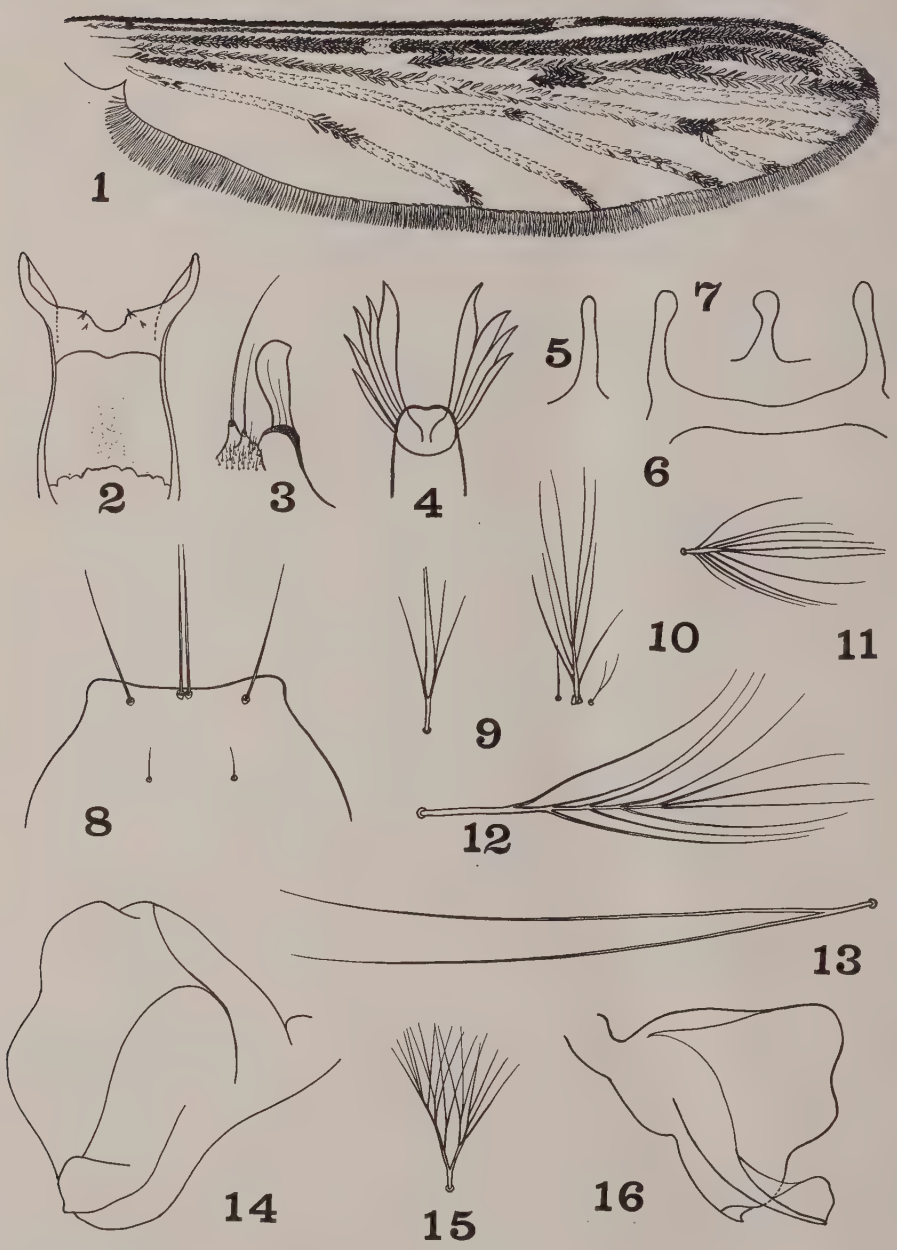
Pupa.—Trumpet (Fig. 14) shallowly emarginate, tragus small, not projecting beyond margin of the trumpet. Postero-lateral spines on abdominal segment II very small, progressively larger on segments III–VII, rounded, and longitudinally striate; with many small branches on segment VIII. Tergites without teeth on posterior margins.

Types.—Holotype: Female, with associated larval and pupal skins, Osmena, Samar, Philippine Islands, September, 1945 (L. E. Rozeboom); reared from eggs deposited by adult female caught in animal-baited trap. *Allotype:* Male, with associated larval and pupal skins, and mounted terminalia. Collection data the same as for holotype. Holotype and allotype deposited in U. S. National Museum, No. 61, 111. *Paratypes:* Larval skins, pupal skins, and adults selected from each of five series of reared progeny; collection data the same as for holotype. Paratypes deposited in the U. S. National Museum, the British Museum, and the Johns Hopkins School of Hygiene and Public Health.

Taxonomic discussion.—In his recent revision of the *umbrosus* group of *Anopheles,* Reid (1950) recognizes nine species. Of these, *A. samarensis* most closely resembles *A. baezai,* and its validity can be established only through the demonstration of constant differences between it and *A. baezai.* The adult of *A. samarensis* differs from that of *A. baezai* by the presence of more distinct pale bands on the tarsi; in the latter species they are absent or indistinct.

The most distinctive feature of *A. samarensis* is found in the single or at most 5-branched outer clypeal hairs of the larva; in this *A. samarensis* differs not only from *A. baezai* but from all other species of the *umbrosus* group. The constancy of this character is shown in Table 1. Not many *A. baezai* were available for comparison, but the specimens examined, as well as the descriptions of *A. baezai* larvae

PLATE I



EXPLANATION OF PLATE

FIG. 1. *Anopheles samarensis*—wing of female.
FIG. 2. *Anopheles samarensis*—pharynx of female.
FIG. 3. *Anopheles samarensis*—lateral view of claspette lobes of male terminalia.
FIG. 4. *Anopheles samarensis*—tip of mesosome of male terminalia.

published by several authors (including Gater, 1934), indicate that the outer clypeal hairs in this species have at least 10 branches (Fig. 15).

This multiple branching of the outer clypeal hairs of the larva was accompanied in three available pupae of Philippine *baezai* by the presence of an elongate lobe or tragus on the trumpet of the pupa (Fig. 16). Two of these pupae (one a paratype of *"gateri"*) came from Palawan, and the third is associated with a *baezai* larval skin, collected by the author at Osmena, Samar. The tragus on the trumpets of these three specimens is somewhat longer than that described for *A. baezai* by Reid (1950). Of 103 trumpets of pupae in the *A. samarensis* reared progeny, none showed more than a very small lobe that scarcely extended beyond the margin of the trumpet. The author also collected an *A. samarensis* adult at Subic Bay, Luzon, P. I., which produced a larva with 5-branched outer clypeals and a pupa without a prominent tragus on the trumpet. These two characters, therefore, appear to be associated, which is additional evidence that the two Philippine forms are genetically different populations.

TABLE 1. *Comparison of branching of outer clypeal hairs of larva of* A. baezai *and* A. samarensis

Species		No. larvae examined	No. hairs counted	Distribution according to number of branches								
				1	2	3	4	5	6–9	10–13	14–17	18–21
baezai- Malaya		2	4								4	
baezai- Philippines		8	14							3	7	4
samarensis Progeny No.	2	7	10	2	1	5	1	1				
" "	9*	1	1		1							
" "	12	18	28	24	4							
" "	13	23	42	34	7			1				
" "	14	4	6	2	3	1						
TOTALS		53	87	62	16	6	1	2				

* The other larvae of this large series appear to have been lost in shipment.

Unfortunately, the decision to consider *A. samarensis* as of specific status must be based upon interpretation of morphological characters. Cross breeding experiments were not possible; however, the fact that the two most distinctive characters occurred uniformly throughout four reared progeny series (and in one available larva and pupa in each of two other series) indicates that these differences are not unstable variations. The single *A. baezai* taken at Osmena, Samar, the type locality of *A. samarensis,* shows that the two forms may exist in apparently the same environment, without intergradation of characters. By definition, two subspecies can not coexist in a single ecological environment, unless, in theory at least, previous isolation permitted the development of some difference in habit which would prevent

FIG. 5–7. *Anopheles samarensis*—lobes of ninth tergite of male terminalia.
FIG. 8 and 9. *Anopheles samarensis*—clypeal hairs of larva.
FIG. 10. *Anopheles samarensis*—prothoracic hairs 1, 2, and 3 of larva.
FIG. 11. *Anopheles samarensis*—hair no. 1 of abdominal segment IV of larva.
FIG. 12. *Anopheles samarensis*—hair no. 6 of abdominal segment IV of larva.
FIG. 13. *Anopheles samarensis*—hair no. 6 of abdominal segment V of larva.
FIG. 14. *Anopheles samarensis*—trumpet of pupa.
FIG. 15. *Anopheles baezai* (Philippines)—outer anterior clypeal hair of larva.
FIG. 16. *Anopheles baezai* (Philippines)—trumpet of pupa.

the two populations from interbreeding. The larval habits of *A. samarensis* are unknown. As all the adult females were captured near the sea coast, it is possible that this species, like *A. baezai,* breeds in brackish water.

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THE TREMATODE FAMILY MICROPHALLIDAE WITH THE DESCRIPTION OF *CARNEOPHALLUS TRILOBATUS* GEN. ET SP. NOV., FROM MEXICO

R. M. Cable and M. L. Kuns[1]
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About 50 specimens of a small microphallid trematode were recovered from an Insect Hawk taken near Palenque in Chiapas, Mexico, in August, 1949. Two additional birds, one from Chiapas and another collected in October, 1950, in southeastern Guatemala, were negative for this parasite. A few specimens were flattened but most were fixed after shaking. About 12 were imbedded in groups and sectioned while the remainder were prepared as whole mounts stained with either hematein or Semichon's carmine.

Although microphallid trematodes may attain sexual maturity in a variety of hosts, their occurrence in raptorial birds is unusual. Since the species at hand is referable to no existing genus, a new one, *Carneophallus* (= "fleshy-phallus") is here proposed to include it and also a species described and allocated to the genus *Spelotrema* by Chen (1944).

Carneophallus gen. nov.

Diagnosis: with the characters of the family Microphallidae (*vide infra*). Cirrus sac absent, well developed seminal vesicle and prostate lying free in the parenchyma. Ventral sucker single, median, situated well within posterior half of body with genital pore to left. Genital atrium large, thin-walled, without pockets, and almost filled with fleshy male papilla which is divided into lobes, one of which is penetrated by the ejaculatory duct. Testes well separated, just posterior to terminations of the short ceca at the level of the ventral sucker. Ovary dextral, immediately anterior to right testis; vitelline follicles in a few large masses forming a cluster on each side posterior to testes; seminal receptacle apparently absent. Uterus not extending anteriorly beyond level at which ceca terminate. Adults in intestine of birds. Life cycle unknown. Includes:

Carneophallus trilobatus sp. nov., type species.

Carneophallus pseudogonotylus (Chen) comb. nov., syn. *Spelotrema pseudogonotyla* Chen, 1944.

Although the lobed male papilla distinguishes *Carneophallus* from all other microphallids, the genus is otherwise identical with certain others. Superficially, the structure of the copulatory organs gives the genital atrium the appearance of that in species of *Levinseniella*. In the latter, however, the wall of the atrium is thickened to form pockets and the male papilla, when present, is small and independent of these pockets. While the papilla in *Carneophallus* is suggestive of the copulatory organ in the genus *Gynaecotyla,* the latter has not only a cirrus sac but also two ventral suckers and the positions of the ovary and genital pore are reversed. Evidently, the genera most closely related to *Carneophallus* are *Spelotrema* and *Microphallus*. These possess a simple male papilla which, however, may be a massive structure. The development of a lobed papilla in a species such as *Spelotrema papillorobusta* Rankin, would result in a form differing in no important respect from those here assigned to the genus *Carneophallus*.

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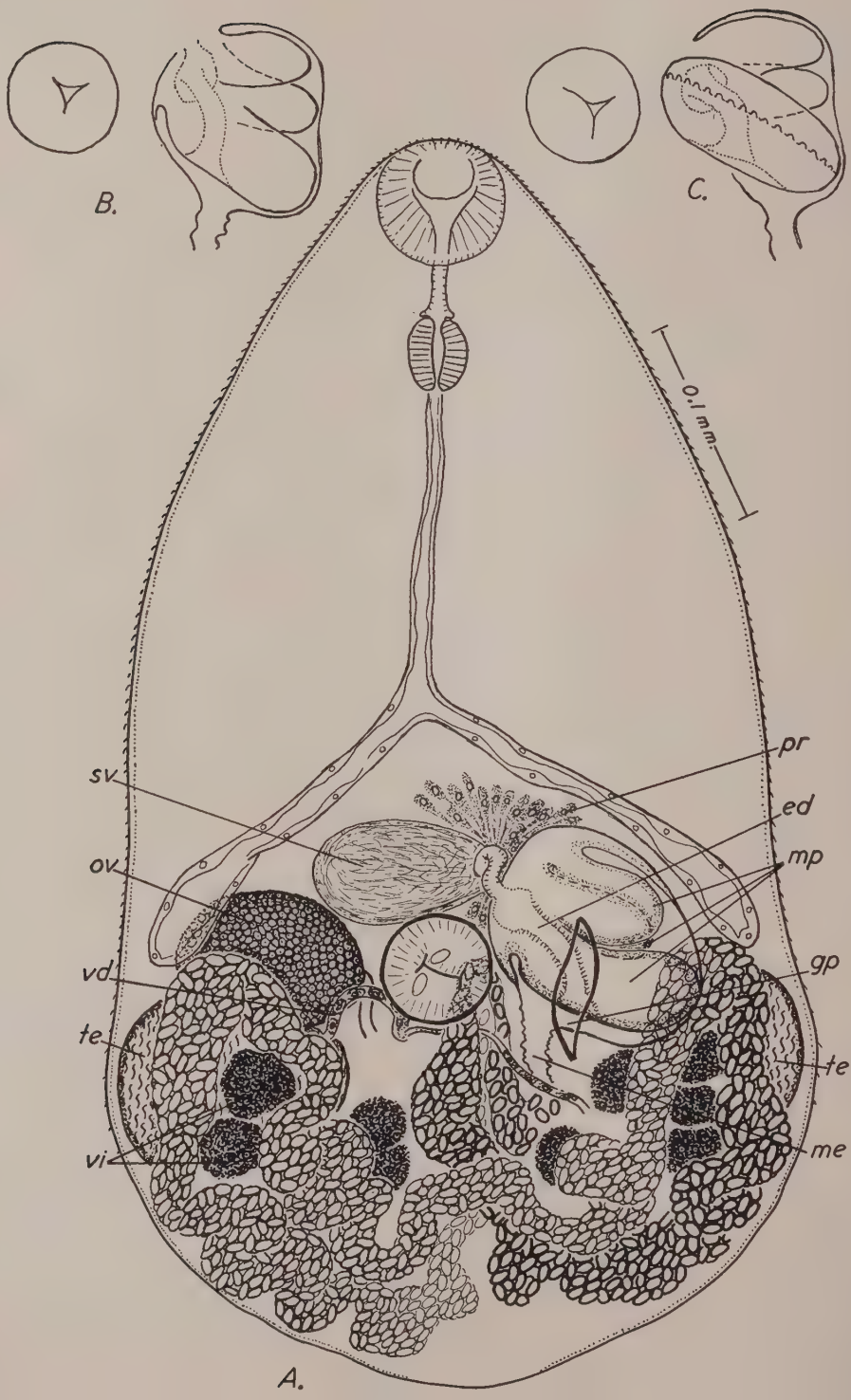
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Carneophallus trilobatus sp. nov. (Fig. 1)
(All measurements in millimeters)

Diagnosis: with characters of the genus. Small, pyriform distomes with a deep longitudinal furrow on the ventral surface in unflattened specimens. Body 0.58–0.61 long and 0.34–0.40 in maximum width, always at the testicular level; sometimes slightly widened at the level of the esophagus. Oral sucker 0.053–0.060 long and 0.060–0.063 wide; ventral sucker 0.056–0.058 in diameter. Prepharynx about length of pharynx which is spherical, measuring 0.028–0.031 in diameter; esophagus long, ceca short, ending at level of ventral sucker, with walls of irregular thickness and usually with somewhat expanded terminations. Testes smooth and spherical, measuring 0.092–0.10 in diameter, to ovoid, measuring 0.102–0.122 in transverse diameter by 0.088–0.092; left testis tends to be more spherical than right. Seminal vesicle variable, ovoid when filled and may exceed 0.095 in transverse diameter by 0.050; prostate well developed. Genital atrium somewhat triangular in outline, conforming in shape with that of the male papilla which is trilobed with two ventral and one more dorsal lobe, all broadly fused at the base and attached to the anteromedian wall of the atrium; posterior-most of the ventral lobes largest, pierced by the broad ejaculatory duct, and bearing a row of minute papillae; genital pore slit-like. Ovary immediately anterior to right testis, ovoid, measuring 0.09–0.10 in transverse diameter by 0.060–0.065. Vitelline ducts unite to right of midline to form a small vitelline reservoir. Uterus extensively coiled, obscuring the testes and vitellaria; metraterm with a thin, wrinkled cuticular lining enters the atrium posterodorsally. Eggs numerous, measuring about 0.008 by 0.015.

Host: The Insect Hawk, *Buteo magnirostris griseocauda* (Ridgway).

Locality: Palenque, Chiapas, Mexico.

Type specimens: Holotype and paratypes deposited in the Helminthological Collection, U. S. National Museum (Holotype No. 37,330; paratype No. 37,331).

Carneophallus trilobatus is readily distinguished from *C. pseudogonotylus,* the only other species in the genus, by having a male papilla with three instead of two lobes. Furthermore, in *C. pseudogonotylus,* the smaller lobe lies nearest the genital pore and guards that opening, whereas in *C. trilobatus,* the largest lobe bearing the male pore occupies that position. In sections of specimens with the male papilla protruded, the lobes are spread fan-wise and the margin of the genital pore is not evident, the wall of the atrium seeming to be continuous with the ventral surface of the body.

Baer (1943) has reduced *Spelotrema* and *Monocaecum* to synonomy with *Microphallus,* pointing out that the only difference seems to be a matter of hosts. He emphasized the fact that microphallid metacercariae develop to a point that only a short time is necessary to become sexually mature after excystment in the intestine of the definitive host. Hence it seemed to him that the worms might persist in unnatural hosts long enough to become fully mature with eggs in the uterus. That a single species may develop to adults in a variety of hosts has been demonstrated experimentally by Rausch (1947) who fed metacercariae of *Microphallus opacus* to a number of animals and recovered adult trematodes from two species of turtles, two snakes, the opossum, and raccoon. Worms failed to develop to maturity in salamanders, frogs, chicks, albino rats and a skunk. Rausch's observations not only support Baer's opinion but also suggest that microphallids are not as host-specific as many trematodes are. There is growing evidence that closely related trematodes may not necessarily have closely related definitive hosts and that separation of spe-

FIG. 1. *Carneophallus trilobatus* drawn by microprojection. A, holotype, ventral view. B, genital atrium showing lobes of male papilla in position usually observed. C, specimen with largest lobe of male papilla occluding genital pore and turned so that the row of minute papillae is visible. Lettering: *ed,* ejaculatory duct; *gp,* genital pore; *me,* metraterm; *mp,* male papilla; *ov,* ovary; *pr,* prostate; *sv,* seminal vesicle; *te,* testis; *vd,* vitelline duct; *vi,* vitellaria.

cies on the basis of host alone is unwarranted. This criterion has been used, for example, in differentiating species and even genera of strigeoids, that otherwise are essentially identical. It may be pointed out that these trematodes, like the microphallids, often have metacercariae that develop to maturity in a short time in the intestine of the definitive host. For that reason, a species might remain in a variety of hosts long enough to become ovigerous. Furthermore, its development in dissimilar hosts might be such as to show sufficient morphological differences to lead to an erroneous interpretation of genera and species.

The microphallids illustrate perhaps better than any other group what seems to be an axiom in the evolution of the digenetic trematodes: that reduction and loss of a protrusible cirrus and the cirrus sac essential to its function are accompanied by the development of accessory structures facilitating copulation and cross-fertilization. This development has assumed a variety of forms in different groups and was responsible for the earlier view that the heterophyids and microphallids are closely related. Certain of the OPECOELIDAE lacking a cirrus sac have acquired an accessory sucker near the genital pore. In the HETEROPHYIDAE, a gonotyl and ventrogenital sac compensate for absence of the usual copulatory structures, while the strigeids have acquired a complex bursa in the absence of a cirrus and cirrus sac which are still present in the closely related cyathocotylids. In the MICROPHALLIDAE, accessory modifications are more diverse than in any of the examples just cited except possibly the heterophyoids. Yet with the exception of the copulatory organs, it would be difficult to find among the DIGENEA a group of comparable size that is more homogeneous in the morphology of its members. Moreover, their close relationship is supported most emphatically by life history studies. For these reasons, the writers do not agree with Baer (1943) in separating them into two families, the MARITREMATIDAE to include those possessing a cirrus sac, and the MICROPHALLIDAE for those lacking that structure. Baer admitted that the two families are closely related. Yet their separation without indicating a category of higher order to express this relationship leaves the two groups of equal rank among a series of families that evidently are interrelated and have been assigned to the superfamily PLAGIORCHIOIDEA. A comparison of the genera Baer has segregated into distinct families convinces the writers that he has ascribed undue significance to the presence or absence of a cirrus sac and has proposed an arbitrary arrangement that does not serve the concept of a natural classification. Among the genera he has allocated to the MARITREMATIDAE, *Gynaecotyla* has the position of the ovary and genital pore reversed in comparison with all other members of either the MARITREMATIDAE or MICROPHALLIDAE. Furthermore, the arrangement of the vitellaria in *Gynaecotyla* is identical with that of the MICROPHALLIDAE, and in the one known life cycle, that of *G. nassicola*, the cercaria is of the Ubiquita type as in the MICROPHALLIDAE and not of the Armata type reported for *Maritrema rhodanicum* by Carrère (1936). In *Microphalloides*, the vitellaria are in the forebody while in *Pseudospelotrema* they are arranged as in the MICROPHALLIDAE; in neither do they have the form characteristic of *Maritrema*.

The copulatory complex in these trematodes seems to be an especially plastic one that has become diverse in structure by evolution in several directions with reduction and loss of the primitive cirrus and cirrus sac. For that reason, we propose that but one family, the MICROPHALLIDAE, be recognized to contain all of these

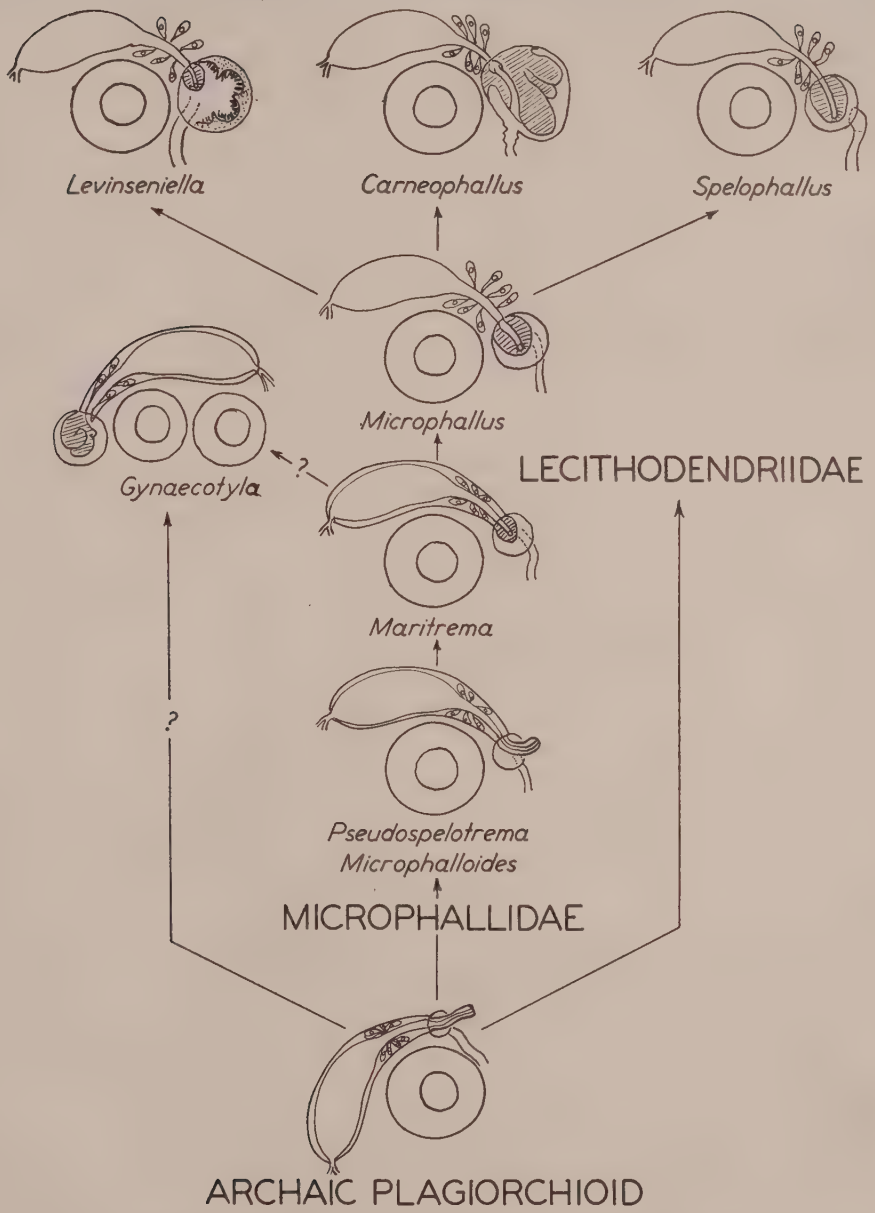

FIG. 2. A concept of phylogeny of the Microphallidae and derivation of copulatory organs in the various genera.

genera. Our concept of their origin and interrelationships is summarized in Fig. 2. As several authors have suggested, the morphology and life histories of the MICROPHALLIDAE justify their inclusion in the superfamily PLAGIORCHIODEA of which the family LECITHODENDRIIDAE would seem to be most closely related to the MICRO-

PHALLIDAE. In proposing the family MICROPHALLIDAE, Travassos (1921) pointed out resemblances suggesting this relationship as have many subsequent authors. Rothschild (1937) was of the opinion that the LECITHODENDRIIDAE differ from the MICROPHALLIDAE in only one very important character, viz., in possessing a small seminal receptacle. Whether this structure is present in the MICROPHALLIDAE or a fertilization chamber has been misinterpreted as such, remains to be demonstrated.

Among the genera here construed as belonging to the MICROPHALLIDAE, *Microphalloides* would seem to be most primitive since it possesses a cirrus sac and protrusible cirrus and distribution of the vitellaria is remindful of the LECITHODENDRIIDAE. The genital pore has shifted to the left side of the ventral sucker where it occurs in all microphallids except species of *Gynaecotyla*. *Pseudospelotrema* is similar to *Microphalloides* except in the extent of the uterus and the position of the vitellaria which is more typical of the group as a whole. In *Maritrema*, the cirrus sac is still present but the protrusible cirrus is replaced by a conical male papilla. This genus seems to us to be the most important one in the evolution of the group since it has acquired the male papilla of the more highly specialized genera but still retains the more primitive cirrus sac. On this fact and the remarkable uniformity of the group in all respects except copulatory structures, is based our opinion that the microphallids should not be separated into families in the manner Baer has proposed. At first sight, the distribution of the vitellaria in *Maritrema* would seem to be quite different from that of all other genera; follicles are confluent both anteriorly and posteriorly, forming a corona encircling the reproductive system. Actually the anterior segment of this ring consists of prominent transverse vitelline ducts and posterior confluence of vitelline masses is not as complete in some species as others. Thus the arrangement of the vitellaria in *Maritrema* does not differ fundamentally from that of other microphallids.

In *Microphallus*, the male papilla becomes further developed than in *Maritrema*, the cirrus sac is lost, and the vitellaria have the arrangement typical of most microphallids. From a condition exemplified at present by *Microphallus*, the group seems to have evolved in several directions: one leading to the genus *Levinseniella* in which the male papilla remains small but the atrium becomes complex in structure; another to *Carneophallus* in which the papilla is lobed but the atrium remains thin-walled; and a third to *Spelophallus* which is identical with *Microphallus* except that the opening of the vagina shifts from its location deep in the atrium to a point near the genital pore. The position of the genus *Gynaecotyla* in this scheme is uncertain. In possessing both a muscular copulatory organ and a cirrus sac, it would seem to be related to *Maritrema*. However, the reversal of the position of the ovary and genital pore, the structure of the copulatory organ and presence of an accessory sucker suggest that this genus may have descended in a separate line from an ancestral form. However, amphitypy with a reversal of organ arrangement is well known in the trematodes and it is conceivable that fixation or establishment of a reversed type under genetic control would be facilitated by their ability of self-fertilization. Thus there would be no great difficulty in deriving the genus *Gynaecotyla* from a type such as *Maritrema*.

It is well established that xiphidiocercariae of the Ubiquita type, in which the ventral sucker and digestive system are undeveloped, are larvae of the MICROPHALLIDAE and Carrère (1936) has reported a cercaria referred to the "Armata" group

for a species of *Maritrema*. It seems very improbable that the larva of *Maritrema* would belong to Cercariae Armatae as divided by Sewell (1922) into two groups, both containing species with flame cell patterns and excretory vesicles that scarcely can be harmonized with what is known of the excretory system in *Maritrema*. Carrère's description is very brief, including little more than the statement that intestinal ceca and a ventral sucker are present. For this reason it may be presumed that the presence of these structures is the chief difference between the cercaria he had and the better known microphallid larvae. It would be especially enlightening to know more about the cercariae of *Maritrema* and especially of the more primitive genera, *Microphalloides* and *Pseudospelotrema*. Such knowledge might confirm, as Carrère's observation suggests, that with modification of the copulatory organs, there is delayed development of the ventral sucker. In this connection, it may be noted that in the OPISTHORCHIOIDEA which lack a cirrus sac and may have highly modified genitalia of a type quite different from that of the MICROPHALLIDAE, the cercaria, like that of microphallids, may lack a ventral sucker or have a very minute one.

None of the existing characterizations of the MICROPHALLIDAE are as complete as present knowledge affords. For that reason, the family is redefined as follows:

Family Microphallidae Travassos, 1921, *char. emend.*
Syn. *pro parte* Maritrematidae Baer, 1943.

Diagnosis: Plagiorchioidea. Small to very small trematodes, pyriform or linguiform in outline, often with strongly concave ventral surface; cuticle usually spinose. Oral sucker simple; one or sometimes two ventral suckers side by side usually in posterior half of body. Prepharynx, pharynx, and esophagus present, the esophagus usually much longer than prepharynx and bifurcating well back in the forebody; ceca short and widespread, rarely extending posterior to ventral sucker and never far beyond that structure. Genital pore lateral to, and separate from ventral sucker, rarely dextral and then when two ventral suckers present. Cirrus sac present or absent, it or seminal vesicle lying transversely anterior to ventral sucker. Male copulatory organ either a typical cirrus or a muscular structure which may be simple, lobed, or complexly formed. Genital atrium small to very large, thin-walled, or thickened and folded to form pockets. Vaginal opening deep in atrium or rarely in its lateral wall near genital pore. Ovary dextral as a rule and sinistral only when genital pore is dextral. Testes symmetrically opposed or slightly diagonal, posterior to ovary and well removed from posterior end of body. Vitellaria usually in two distinct clusters most often posterior to testes, rarely varying from that position to a point anterior to ventral sucker; sometimes band-like, confluent posteriorly and with prominent ducts uniting anteriorly, the whole forming a corona encircling most of the reproductive system. Laurer's canal probably always present; seminal receptacle present or lacking. Uterus with coils from posterior end of body to, or rarely well anterior to, testis on each side. Eggs small and numerous. Excretory pore at posterior end of body, vesicle broadly V-shaped with a main excretory canal joining the tip of each arm, extending to about the level of ventral sucker and then receiving an anterior and posterior collecting tubule. Adult excretory pattern $2[(2+2)+(2+2)]=16$ flame cells. Larva a xiphidiocercaria, usually with undeveloped ventral sucker (Ubiquita type) or with sucker present (Armata (?) type), developing in sporocysts in prosobranch gastropods; encyst, as a rule, in crustaceans but a free-swimming period may be suppressed with encystment in the molluscan host. Metacercariae develop to an advanced stage, requiring but a short time to become ovigerous after excystment in the definitive host. Adults in the intestine of all classes of vertebrates. Includes the following genera:

Microphallus Ward (syn. *Spelotrema* Jägerskiöld; *Monocaecum* Stafford).
Levinseniella Stiles and Hassall.
Maritrema Nicoll.
Spelophallus Jägerskiöld.
Microphalloides Yoshida.
Pseudospelotrema Yamaguti (syn. *Maritreminoides* Rankin).
Gynaecotyla Yamaguti (syn. *Cornucopula* Rankin).
Carneophallus gen. nov.

SUMMARY

Carneophallus trilobatus gen. et sp. nov. is described from a Mexican Insect Hawk, *Buteo magnirostris griseocauda,* and *Spelotrema pseudogonotyla* Chen is transferred to *Carneophallus* with *C. trilobatus* as type species. The family MICROPHALLIDAE is discussed and redefined, retaining in it genera possessing a cirrus sac as well as those lacking that structure.

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IN MEMORIAM

Marcos A. Tubangui (1893–1949)

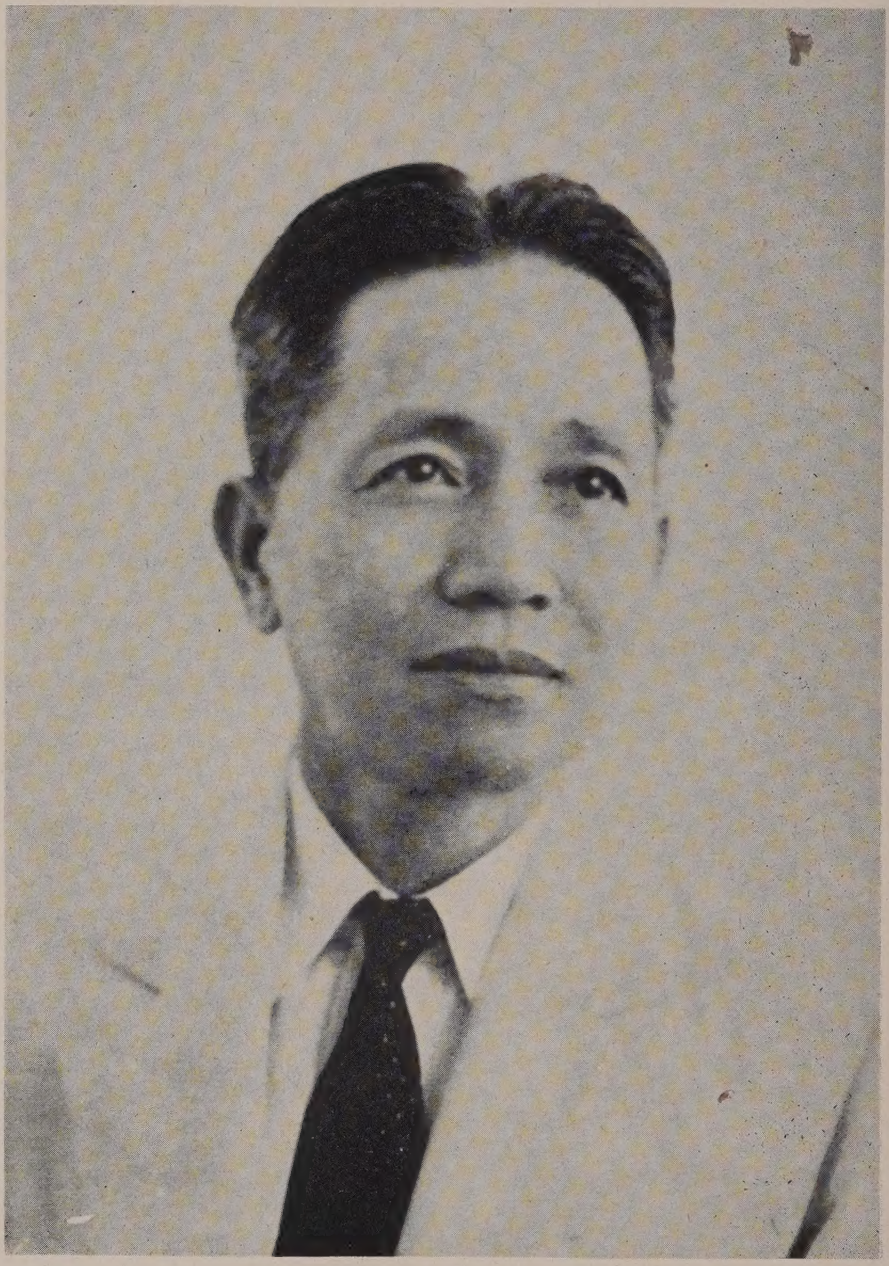
Marcos Angeles Tubangui was born at Porac, Pampanga, on the island of Luzon in the Philippines on April 25, 1893. He was graduated from the College of Veterinary Science of the University of the Philippines with the D.V.M. degree in 1918. For a time after his graduation, he served as a veterinarian with the Philippine Bureau of Agriculture.

Appointed as a Fellow at the University in 1919, he went to the United States where he registered as a graduate student in the New York State Veterinary College, Cornell University, Ithaca, New York. He spent the academic year 1919–20 engaged in advanced work in physiology and pharmacology. The degree, Master of Science, was conferred on him in 1921.

In the fall of 1920, he enrolled in the graduate school at the University of Illinois where he remained for a semester working in the field of helminthology under the late Professor Henry B. Ward. Leaving Illinois at the end of one semester, he moved to the Zoological Division of the Bureau of Animal Industry in Washington, D. C. Here he was associated with an active group of parasitologists and zoologists, among whom were the late Brayton H. Ransom, Maurice C. Hall, Charles Wardell Stiles and Nathan A. Cobb. He also met a number of visiting parasitologists; among others, R. T. Leiper, W. W. Cort and James E. Ackert. It was during his stay in Washington that he prepared his first helminthological paper for publication. A brief visit to the Marine Biological Laboratory at Wood's Hole, Massachusetts, was crowded into his sojourn in the United States. The contact and association with a large number of prominent parasitologists, especially in Washington, did much to influence his activities in later years. The variety of his researches is evidence of interests aroused by some of these early contacts.

Returning to the Philippines in 1921, he was appointed instructor, and later, assistant professor, in the College of Veterinary Science of the University of the Philippines. He taught physiology and assisted Dr. Benjamin Schwartz, then in charge of the Department of Veterinary Parasitology. From this association, several scientific publications resulted. Upon Dr. Schwartz' return to the United States, Dr. Tubangui succeeded him as chairman of the department. This background of experience and personal contacts, together with circumstances permitting investigation on parasitological problems, led him to continue in this field which was, in large measure, unexplored in the Philippines.

In 1930, he resigned from the University to accept a position as parasitologist in the Bureau of Science. Ultimately, he became chief of the Division of Biological Research. His more important contributions in parasitology were made during this period and established him as one of the foremost workers in the field. While his research activities covered a variety of subjects, his published papers, many in collaboration with associates, reflect his deep interest in the trematodes. Much of his work dealt with taxonomy, but other aspects are well represented. In particular, his attention to trematode life cycles deserves special mention. With a colleague, he elucidated the life cycle of *Echinostoma ilocanum,* a not infrequently encountered



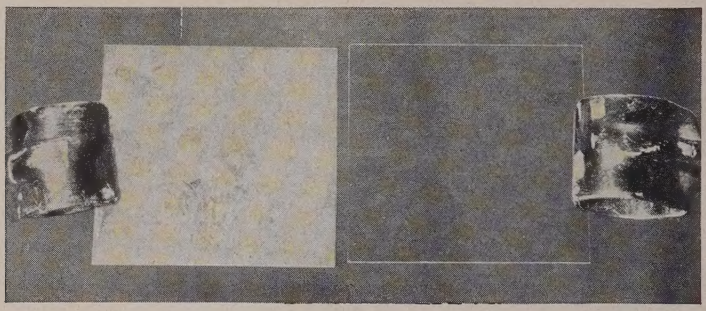
MARCOS A. TUBANGUI

human parasite in certain sections of northern Luzon. He studied the incidence and distribution of *Schistosoma japonicum* and discovered the snail intermediate host which served the parasite in the Philippines. He likewise discovered the crustacean intermediate host of the lung fluke, *Paragonimus,* which occurs in several local areas in Southern Luzon. His bibliography involves 67 titles, all but four of which deal with parasitological subjects.

Early in 1948, he was appointed head of the Department of Parasitology and Professor of Helminthology in the Institute of Hygiene and Professorial Lecturer in the Post Graduate School of Medicine, University of the Philippines. In addition to his academic duties, he was initiating young associates in the field of research as well as continuing work on several research projects interrupted during the war years.

As a colleague, Dr. Tubangui was kindly, modest and cooperative. He well-deserved the high esteem in which he was held by his country-men. Following the liberation of the Islands from the Japanese occupation, he was most helpful to personnel of the United States Armed Forces. Many of the younger American parasitologists then assigned to duty with Medical Department units sought him out for information, advice, and assistance. His professional ability and good judgment as well as his capacity for friendship won him a host of new friends among them. They, no less than his associates at home and old friends the world over, will recognize that the Philippines have lost their most distinguished parasitologist in his death on October 26, 1949.

LOPE M. YUTUC



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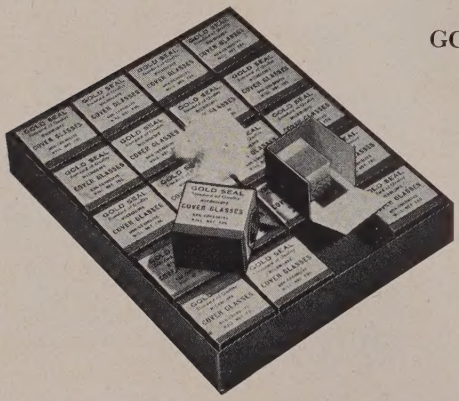

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